MANAGEMENT OF SPIONID MUD WORM INFESTATIONS OF TASMANIAN CULTURED ABALONE

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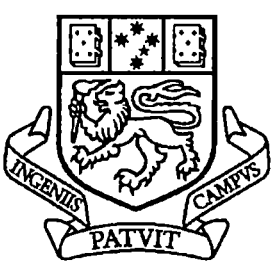
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy



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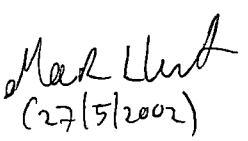

(27/5/2002)

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ABSTRACT

Stock losses in the range 50-90% were recorded at experimental and pilot scale sea-based abalone farms in southern Tasmania in the mid-1990's. These were associated with spionid polychaete "mud worm" infestation, especially *Boccardia knoxi*. The overall aim of the multi faceted research presented here was to minimise the effects of spionid infestation. Studies of reproductive biology indicated initial infestation with *B. knoxi* could be delayed by placement of stock after the spring planktotrophic dispersal phase. This would also reduce infestation by a second species *Polydora hoplura*. Fieldwork during 1998-2001 indicated that large settlements of spionids might be relatively uncommon. Testing of 16 chemical/drug agents and freshwater bathing as a treatment for mud worm infestation failed to yield a useful candidate. Agents lacked penetration into shell burrows or were harmful to abalone at levels sufficient to kill spionids *in situ*. Air exposure of abalone for 2-4 hours at humidity less than approximately 63% was highly effective as a spionid treatment, especially in the first 6 months post infestation. Assessment of risk factors associated with spionid settlement found that elevated levels of spirorbid polychaetes enhanced mud worm colonisation. Stock size, rearing vessel design and position in the water column also led to differential spionid settlement outcomes. Spionid infestation was associated with a reduction in: growth, % flesh weight, protein and carbohydrate reserves and with an increased respiration rate. Major histological changes were elevated levels of brown pigment in the right kidney and digestive gland consistent with mobilisation and consumption of energy reserves. The ranges in levels of sodium, potassium, calcium, magnesium, chloride, copper, glucose and protein in haemolymph were established for apparently healthy abalone and spionid infested animals.

ACKNOWLEDGMENTS

I wish to acknowledge the assistance of the following people and organizations for their contributions to the undertaking of this research:
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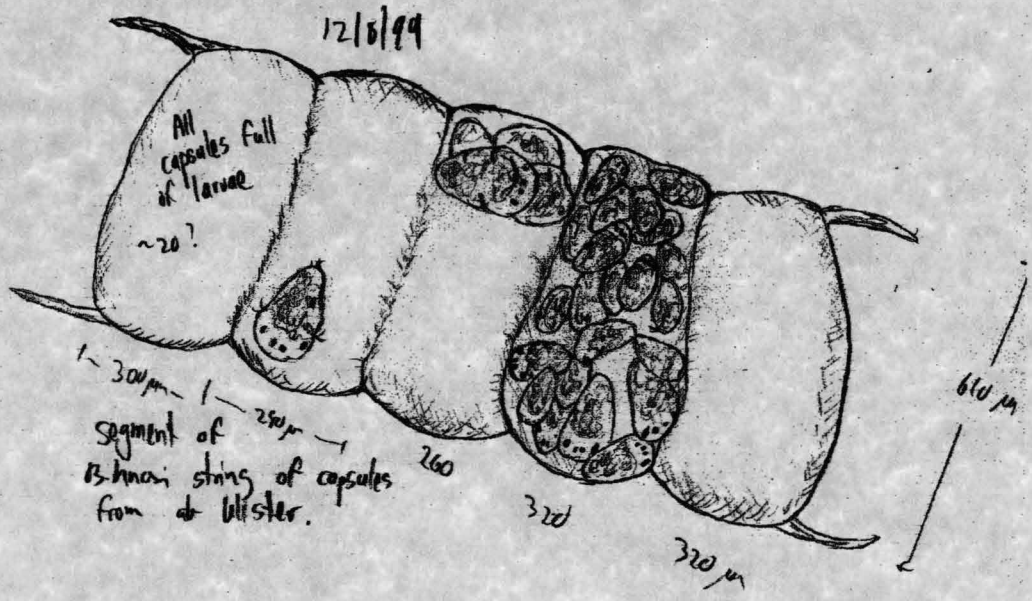
LIST OF ABBREVIATIONS

ANOVA	analysis of variance
°C	degrees celsius
cm	centimetre
d	day
g	gram
h	hour
H & E	haematoxylin and eosin
kg	kilogram
l	litre
min	minute
mg	milligram
ml	millilitre
mm	millimetre
µm	micrometre
PAS	periodic acid-Schiff
PPM	parts per million
SD	standard deviation
SE	standard error
SGR	specific growth rate
SSDR	subjective shell damage rating


12/8/99
All capsules full of larvae
~20?
~300μm
~250μm
Segment of B. hmai string of capsules from the blister.
260
320
320 μm
610 μm

Frontispiece: section of *Boccardia knoxi* egg string

Chapter 1
INTRODUCTION

1.1 Rationale

Abalones are marine gastropod molluscs of which there are approximately 100 species, worldwide (Fallu 1991). The larger, generally temperate species constitute a valuable marine resource. Abalone are prized for the for their large fleshy foot and adductor muscle and important fisheries have existed in Australia, China, Japan, Mexico, New Zealand, South Africa and the United States (Hahn 1989). Decline of some of these fisheries by the 1980's and continued demand led to interest in propagation of abalone including reseeding programs and captive culture methods. Australia accounts for 60% of world fishery production and prices have been steady at Aus$35-40 kg^{-1} for several years (Fleming and Hone 2001).

Abalone culture in Australia commenced in the mid-1980's with land-based farms in Tasmania and South Australia. The primary culture species are the blacklip abalone *Haliotis rubra* Leach and the greenlip abalone *H. laevigata* Donovan. In the mid-1990's there were five sea-based experimental or pilot-scale farms in southern Tasmania. By 1996 these farms had experienced stock mortality levels in excess of 50% which were associated with blisters on the inside of the abalone shell. Both greenlip and blacklip abalone were affected as were hybrids of the two species. Preliminary investigation showed that two spionid polychaete "mud worm" species were present within the blisters of infested abalone. Mud worms are noted pests of farmed bivalve molluscs and consequently considerable research exists on their impact on this group (Blake and Evans 1972, Lauckner 1983).

The spionid species present in the shells of live and deceased abalone were identified as *Polydora hoplura* Claparede and *Boccardia knoxi* Rainer. The former species was previously recorded from Tasmanian farmed and wild oysters (Wilson et al., 1993) but *B. knoxi* was only previously known from New Zealand (Rainer

1973, Read 1975). This latter species was the dominant spionid infesting the stock at the largest culture facility and there was some concern that the destructive *B. knoxi* was an introduced species.

By November 1997 when this study commenced, some marine farms in southern Tasmania had abandoned abalone culture trials or relocated facilities and all farms had discontinued transfers of new stock to their leases. It was apparent that a loss of confidence in sea-based abalone farming had occurred and strategies to minimise the economic impact of spionid infestation would be required before further investment could occur.

1.2 Aim

The overall aim of the research was to minimise the economic impact of spionid infestation on Tasmanian sea-farmed abalone. This was to be achieved by investigation of spionid ecology, reproductive biology and epidemiology in relation to interactions with abalone. By this means preventative strategies and direct treatments would be developed. Additionally, the affects of spionid infestation on abalone health would be characterised, contributing to the knowledge base on abalone health generally.

1.3 Research approach

A multi-faceted research approach was used to achieve the aim stated above. The biology of the spionid species involved with the southern Tasmanian stock mortalities was investigated. Emphasis was on the study of *B. knoxi*, with reference to reproductive biology, larval dispersal and timing of settlement. The intention was to formulate strategies to avoid or minimise transmission of larval spionids to abalone (Chapter 3). Options for the treatment of abalone that become mud worm infested are explored in Chapters 4 and 5. These include experiments on chemical agents with a history of use in aquaculture and traditional terrestrial animal production as anti-parasiticides.

Environmental treatments such as fresh water bathing and air exposure previously used to combat mud worms in oysters are also assessed. The stock health and safety implications associated with use of the most promising spionid treatments are explored in some detail. Experiments were conducted to identify risk factors that may contribute to the extent of spionid infestation. These included, stock and environmental characteristics such as: size, shell characteristics, host species and design of rearing containers. This aspect of the research is presented in Chapter 6. Mud worm infestation was quantified in terms of spionid numbers and blister coverage and the effect of these measured parameters on growth and mortality. Other aspects of abalone health relating to condition indices, physiology, tissue chemistry and histology were also investigated. The health effects of mud worm infestation and the consequences for farmed production are reported in Chapters 7 and 8.

1.4 General background: spionid biology and impacts

Mud worms belong to the family Spionidae, one of the largest families of marine annelid worms in the class Polychaeta. The family is very common in all marine environments and its members include free-living forms in sand and mud as well as species that build permanent burrows in soft substrates (Fauchald 1977). Several genera of spionids, including *Boccardia* and *Polydora*, are capable of boring in calcareous substrates including the shells of molluscs. Boring is achieved by the production of acid secretions (Zottoli and Carriker 1974, Almeida et al. 1996). The name "mud worm" may derive from the often-muddy appearance of blisters formed in molluscs as a result of burrowing activity.

Because of the economic importance of oyster cultivation worldwide, there have been many studies on spionid impacts (see reviews by Blake and Evans 1972, Skeel 1979, Lauckner 1983, Handley 1997). In Australia, oyster culture industries have been severely impacted in the past. The earliest investigations of mud worm infestations were described by Whitelegge (1890). From about 1870 infestations in NSW were so severe that the industry changed to its production mode from sub-tidal dredging to the present intertidal culture system (Smith 1984, Nell and Smith

1988). By about 1900 mud worm infestation in Queensland had devastated sub-tidal production in that state (Smith 1982, Nell and Smith 1988). Potential treatment of spionid infestation in oysters has been previously investigated (Whitelegge 1890, Korringa 1952, Mackenzie and Shearer 1959, Bailey-Brock and Ringwood 1982, Nel et al. 1996). The species most often associated with damage to commercially important bivalve species are *P. ciliata* Johnston, *P. hoplura* and *P. websteri* Hartman (Blake and Evans, 1972).

There is considerably less literature on the incidence and effects of spionids in abalone. Mud worms have been reported from wild haliotid species by: Sinclair (1963), Shepherd (1973), Clavier (1989), Horne (1996) and Grindley et al. (1998). In Japan, Kojima and Imajima (1982) found that more than 10 *Polydora* per shell significantly decreased the flesh weight of wild *H. diversicolor* Reeve. References to mud worm incidence in cultured abalone have been fewer. Ruck and Cook (1999) note that *Polydora* is a potential problem in *H. midae* and McCormick (1999 pers. comm.) described incidents of severe mud worm infestation in *H. discus hannai.*

In addition to impacts on molluscs there have been many studies on the reproduction of spionids (Wilson 1928, Hopkins 1958, Dorsett 1961, Blake 1969, Anger et al., 1986). A dichotomy in larval feeding and dispersal modes have been described (Radashevsky 1994) where spionids either obtain nutrition from yolk supplies (lecithotrophy) growing to a relatively large size with reduced dispersal capabilities or feed in the plankton (planktotrophy) and potentially disperse widely. These variations in spionid reproductive strategies may result in differential impacts on host mollusc species.

1.5 The original Tasmanian abalone mortality episode

During the period of 1995-1997 various abalone culture facilities within Tasmania contacted the State Government Fish Health Laboratory to report cumulative mortality levels of up to 50% or more associated with mud worm blisters. Heavily blistered abalone were examined from four sea-based grow out farms in the south of the state and similarly infested scallops from another farm. Mortality at one of the southern sea farms was in excess of 95%, involving the loss of more than 30 000 animals by late 1997 (O'Brien 2001, pers. comm.). Hindrum (1996) reported approximately 40% mortality between November 1995 and April 1996 at one site. Infested abalone at the southern farms, mainly located in the D'Entrecasteaux Channel, were infested with both *B. knoxi* and *P. hoplura* but no attempt was made to quantify spionid numbers until late 1997. Three of the farms involved were: Huon Aquaculture Company Pty Ltd, Aquatas Pty Ltd and Tasmanian Tiger Abalone Pty Ltd. The two former farms subsequently became study sites for this research and a small number of remnant animals from Tasmanian Tiger Abalone were made available for study. Mud worm associated mortality was also reported from a sea-based farm outside the south of the state and the same two spionid species recovered from blisters. A further history of mud worm associated mortality was reported in remnant 5-6 year old, long term infested stocks at an east coast, land-based farm. Samples from this source revealed that *P. hoplura* but not *B. knoxi* was present in severe blisters. Samples obtained from a land-based farm in South Australia also showed solely *P. hoplura* infestations in blistered older stock with a history of mortality. Throughout this thesis farm names have been used where permission has been granted. Where anonymity was preferred, general location is provided and where necessary farms are referred to as farm 1, farm 2 etc.

Some mortality records for the period of June 1994 to April 1996 were available for Huon Aquaculture Company stock (Appendix 1A) and showed a high death rate during the austral summer 1995/1996. Infested stock from this source examined in 1996 had extensive blister damage that was associated almost exclusively with *B. knoxi* infestation.

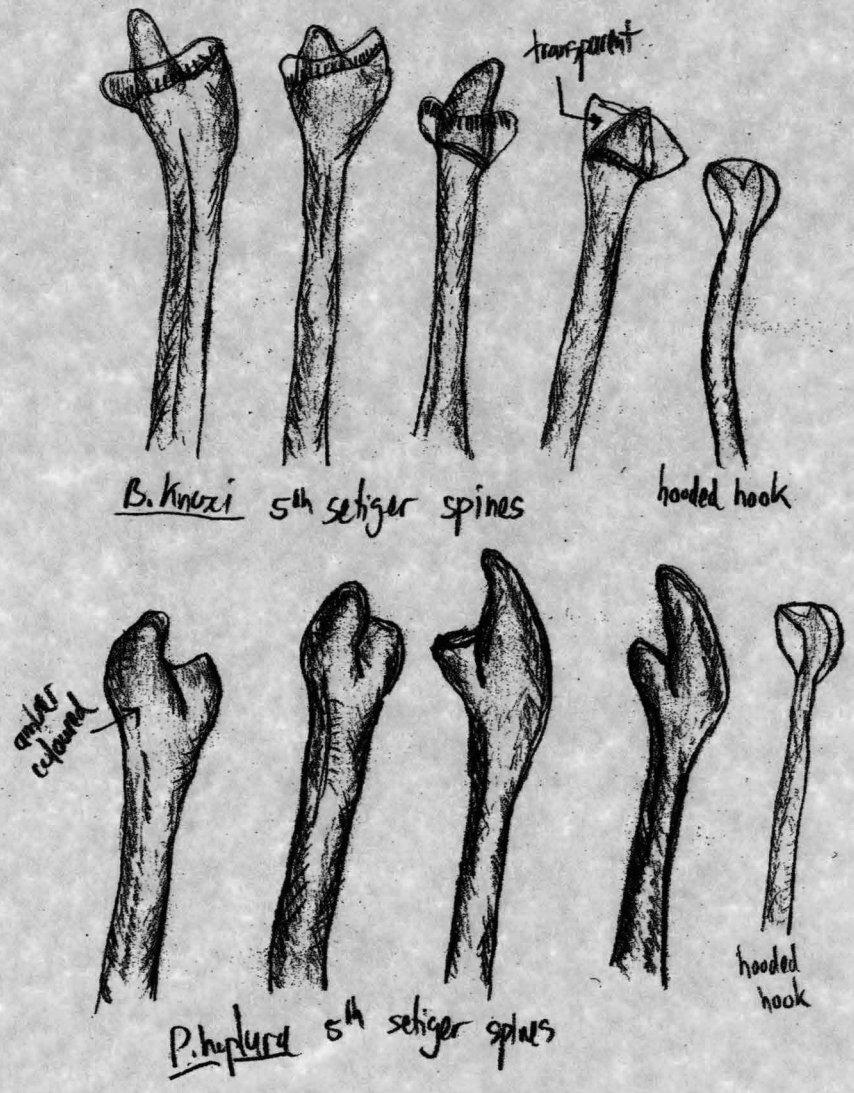
The ventral surface of many shells was characterised by thick, soft, blisters devoid of nacre that apparently failed to heal. By 1997 approximately 1000 shells of dead and mud worm infested live stock remained and these were made available for experiments on spionid treatment options (Chapters 4 and 5). A subjective shell damage assessment was made on a sample of these abalone (Appendix 1B) using the method described in Chapter 2. An estimate of spionid infestation made using the method developed by late 1997 (Chapter 2) found a mean *B. knoxi* count of 36.5 (SD=19, n=40). Mean percentage flesh weight of remnant heavily blistered stock from Tasmanian Tiger Abalone was 52.6% (SD = 3.1%, n=10).

1.6 Previous and preliminary data on spionids in Tasmanian molluscs

In addition to abalone, mortality episodes involving spionid blisters in farmed scallops and Pacific oysters *Crassostrea gigas* Thunberg were also reported to Tasmanian aquaculture health authorities in the mid-1990's. Annual data on blister prevalence in farmed oysters was collected by the Fish Health Unit as part of a surveillance program (Appendix 1C). These data indicate that blister rates for oysters may vary considerably year to year. Additionally, the surveillance program showed the southern region of the state where affected abalone farms were also located had consistently higher levels of oyster blistering than other regions of the state (Unpublished DPIWE Fish Health Unit data). Wilson et al. (1993) found three species of spionids in Tasmanian oysters. In order of prevalence these were *P. websteri*, *P. hoplura* and *B. chilensis* Blake and Woodwick. Although *B. knoxi* was not found in the survey by Wilson et al. (1993) its presence at that time cannot be ruled out since most oysters surveyed were intertidal and *B. knoxi* appears to have a sub-tidal distribution in New Zealand (Handley 1997).

Following the identification of *B. knoxi* in cultured abalone stock Tasmanian aquaculture health authorities commenced sampling of wild abalone populations. Surveys showed the presence of *B. knoxi* in wild stocks from the south, south-east, and east coasts but not in a small number of samples from the north-west (DPIWE unpublished data). No mud worm associated mortality has been reported.

Re-examination of shells from wild abalone collected by abalone fishery research staff at DPIWE Taroona Laboratories over a number of years showed that shell damage in wild stocks was common (Appendix 1D). Most of the damage was attributed to spionid polychaetes but boring sponge damage was also present in many shells. Anecdotal evidence from abalone divers and processors suggests that there are populations of stunted abalone with shells considered too damaged to be used for the jewellery trade. These are typically located in very sheltered area such as the lees of islands and peninsulas. Analysis of samples from such areas present in the shell collection at the Taroona Laboratories indicated stunted abalone had higher rates of shell damage than did non-stunted larger abalone (Appendix 1E).


transparent
B. knuzi 5th setiger spines
hooded hook
amber coloured
P. hupluru 5th setiger spines
hooded hook

Frontispiece: diagnostic 5 th setiger spines from 2 mud worm species

Chapter 2
MATERIALS AND METHODS

2.1 Experimental Animals

The majority of abalone used in the research were blacklip (*H. rubra*) stock from a land-based culture facility located on the east coast of Tasmania (farm 1). Many abalone used in experiments were from a year class spawned in the summer of 1997/1998. These animals were approximately 20 mm when first transfers (referred to as intake groups or cohorts) to study sites were made in spring 1998. By late 1999 when the last intake of this age cohort was used the abalone had grown to approximately 40 mm. The subsequent year class (summer 1998/1999 spawning) was used in December 1999 and 2000. No evidence of any mud worm species was seen in these year class stocks during the research.

Another important experimental group from this farm was a remnant population that were spawned in 1995 and were approximately three years old in mid 1998. These abalone had a low incidence (< 5% of population) along with a low severity (generally < 3 worms) of *B. knoxi* infestation. This group was used in some treatment experiments as described in Chapters 4 and 5 using the presence of characteristic *B. knoxi* chimneys to select for infested animals. Abalone free of *B. knoxi* infestations were selected from this group for use in some transfers to study sites. Animals were chosen based on a lack of spionid chimneys and were air-dried to kill any pre-adult or undetected infestations (Chapter 5).

Where abalone other than blacklip stock were used in specific experiments, or animals were obtained from culture facilities other than farm 1, this is noted in the appropriate section of the thesis.

2.1 Study Sites

A field study site was located in North West Bay at Simmonds Point (DPIWE Farm number 154) and owned by Aquatas P/L, (Figures 2.1 and 2.2). The site is 17-18 m deep, with sediments described as ranging from very fine dark brown sand to muddy sands dark grey in colour. The average current speed is 20 $cm.s^{-1}$ and the tidal range 1.2 m (from D'Entrecasteaux Channel Marine Farming Development Plans for Tasmania, February 1997). Aquatas was predominantly involved in salmon farming during the course of the research but had investigated sea-based abalone farming in the early and mid-1990's. This previous history of mud worm infestation prompted the use of the site for the current research. Abalone were originally housed in culture vessels hung in 3-4 m of water from a barge on the lease but were later moved to a long line 200 m north of the barge.

A second southern Tasmanian study site was located in the Huon River at Hideaway Bay (DPIWE Farm Number 93) and belonged to the Huon Aquaculture Company P/L (hereafter referred to as Huon Aquaculture)(Figures 2.1 and 2.2). The environment is estuarine with a pH range of 7 - 8 and variable salinity. Average monthly temperature range is 12-19 °C. Depth is 6-10 m, with current speed range of 5-20 $cm.s^{-1}$. The sediments are described as sandy to rocky (data from Draft Plan for Huon River and Port Esperance, November 1994). During the research this was predominantly a salmon farming company but with an interest in shellfish including Pacific oysters and abalone. The site was chosen because of its past history of mud worm infestation in abalone. Abalone were housed in culture vessels suspended 3-4 m beneath an empty salmon cage moored in 10 m of water.

A third site was located on the east coast of Tasmania and belonging to Tasmanian Scallops P/L (Figure 2.1). Stock were reared on a commercial scale at this site, suspended from longlines in 20 m of water. The Marine Farm Plan for Maria Island and Mercury Passage, which includes this site, states that the water is 25-35 m deep with bottom type predominantly coarse sand ranging through to fine sand with coarse shell grit. The site is relatively exposed and the dominant current direction is south to north.

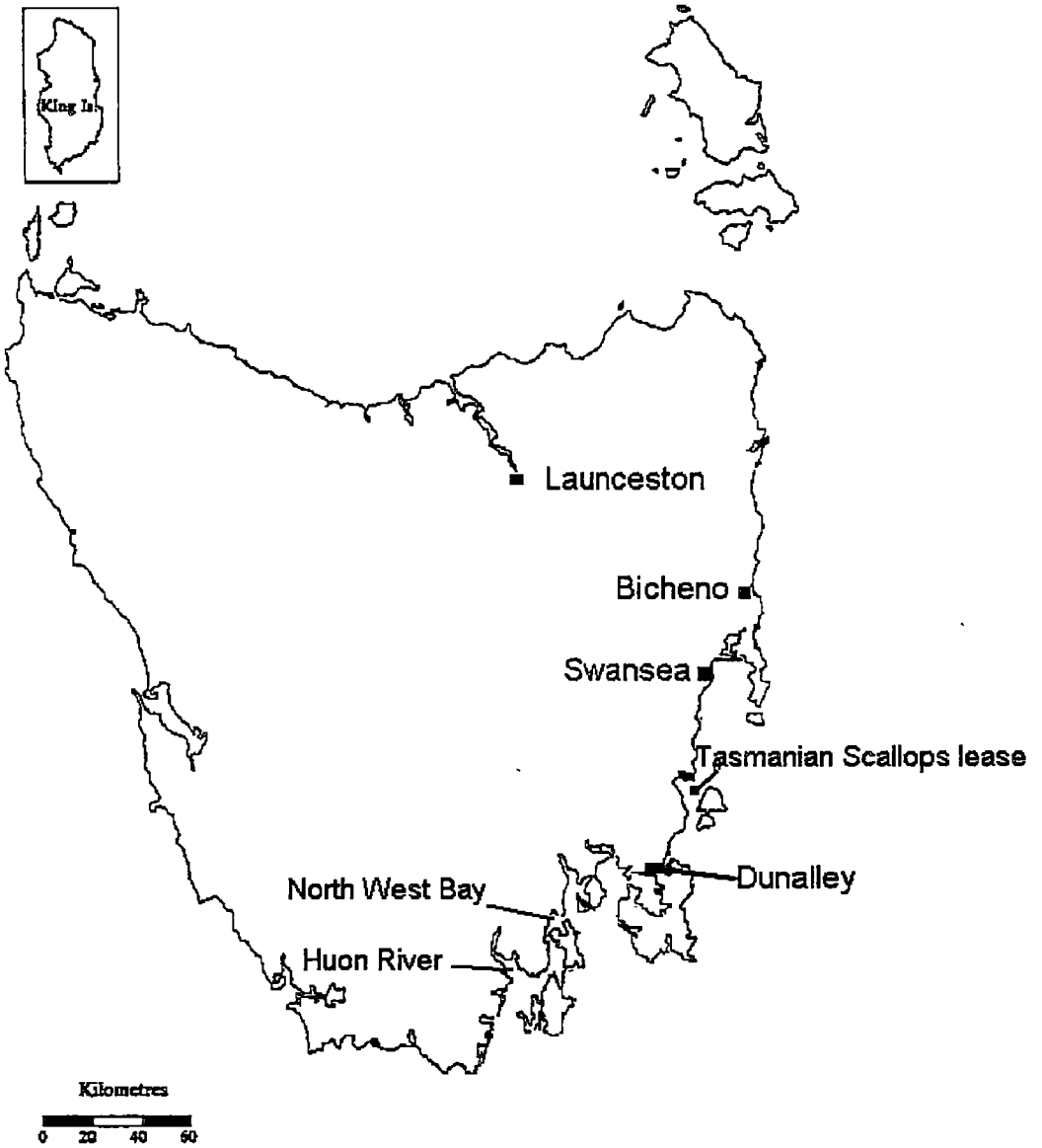


Figure 2.1 Map of Tasmania showing study sites and source farms

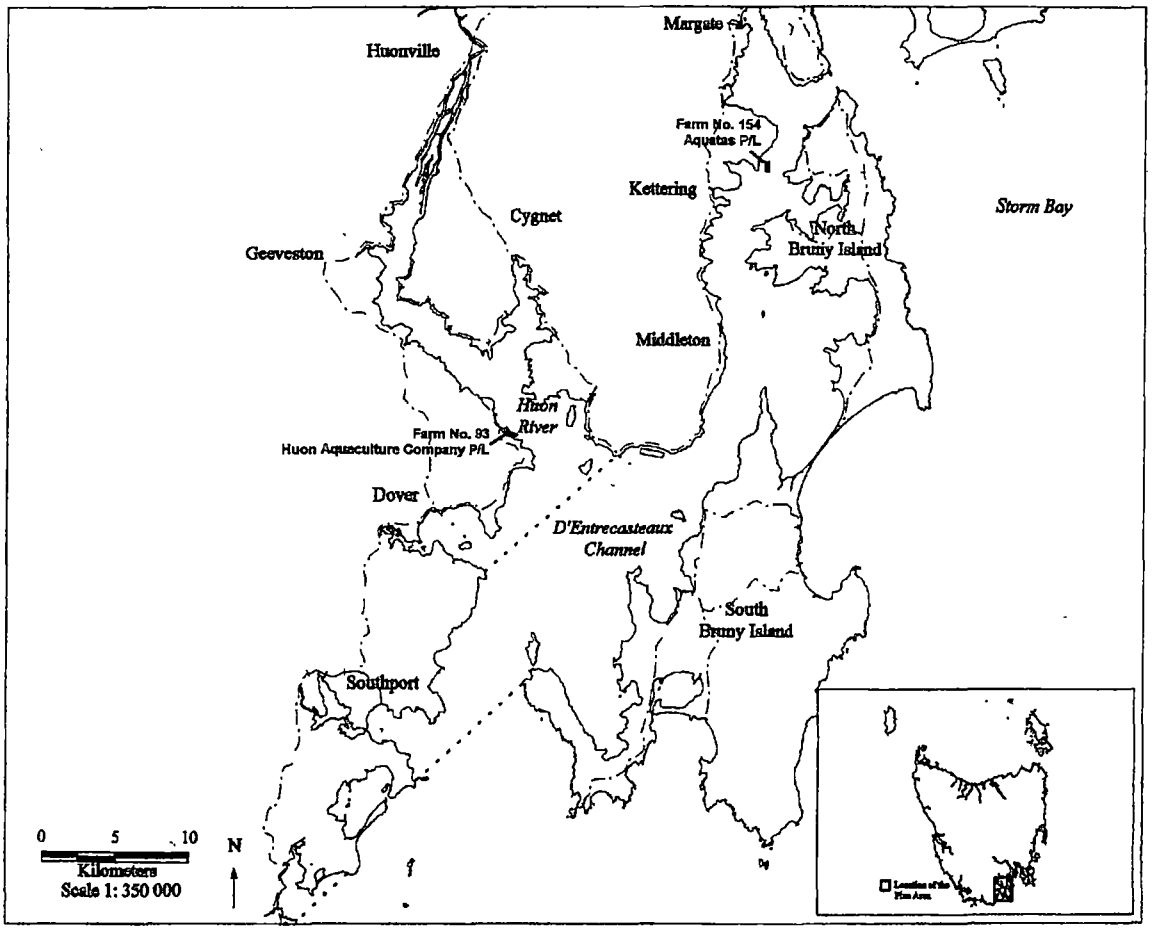


Figure 2.2 Map of D'Entrecasteaux Channel, southern Tasmania showing study sites at Huon Aquaculture Company P/L and Aquatas P/L.

2.3 Holding Conditions

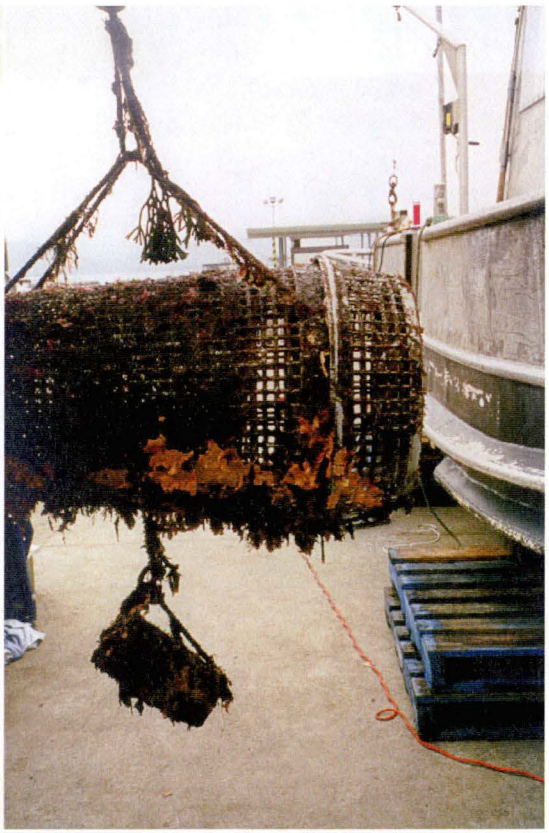
Field Trials

Various types of rearing vessels were used to contain abalone during the research. When mud worm infestations were originally recorded, abalone at several culture facilities were held in vessels constructed from 25 cm PVC pipe fitted with 6 mm mesh ends. These were referred to as "tubes" (Figure 2.3A) and measured 1.5 m in length. Monthly intakes of "clean" abalone were housed in tubes from August 1998 to November 1998. Most abalone were eventually removed from tubes and housed in other types of rearing containers described below. This was because there were insufficient tubes to house all the abalone required for the research and because tubes were relatively expensive to manufacture and heavy to handle.

Nearly all intakes of abalone subsequent to summer 1998/99 were housed in "basket type" culture vessels: modified polyethylene laundry baskets fitted with oyster mesh and plastic inserts to increase mesh-free substrate area (Figure 2.3B). The baskets were approximately cylindrical, 800 mm high with a diameter of 400 mm. A third type of rearing vessel was the Aquatek Aquatray® (Figure 2.3C) measuring 900 mm on each side and 10 mm deep. Bases of these trays were solid with a 12 mm mesh lid and sides. Abalone maintained in the long term were transferred to Aquatrays® from tubes and baskets in late summer 1999 and 2000 respectively: grouping stocks with similar levels of spionid infestation in a smaller number of rearing vessels and reducing maintenance time.

Abalone held at the southern study sites were generally treated in the same way as when the original mud worm problems arose in the mid to late 1990's. Feeding was weekly or bimonthly using Adam and Amos (Mt. Barker, S. Australia) sea cage diet, a formulated abalone food.

A. Tube



B. Basket

C. Tray

Figure 2.3 Abalone containment vessels

Laboratory Holding Conditions

Abalone were moved from field sites to the Animal Health Laboratory (Mt. Pleasant Laboratories, Department of Primary Industry Water and Environment, Launceston, Tasmania) for analysis. Animals were transported in seawater to minimise stress to abalone and polychaetes. Abalone were held for no more than two weeks in a 650 l, recirculating system. This consisted of a fibre glass tank with a conical base and a 400 l sump containing 50 l of "bioballs" as a biological filter medium. The system was aerated and temperature control maintained by use of a 250 W aquarium heater and/or reverse cycle air conditioning. Water temperature was maintained at that of the study sites (±2 °C). Ammonia and nitrite measurements were made approximately monthly using Aquasonic (Ingleburn, NSW) aquarium test kits. Similarly, pH and salinity were measured monthly using Sigma (range 4.5-10) indicator paper and a Shibuya S-10 refractometer, respectively. Ten to twenty percent of tank volume was exchanged on a weekly basis. Abalone samples were separated within the system by placement in mesh bags or plastic aquaria with mesh lids through which air stones were fitted.

2.4 Determination of Polychaete Species

Spionid species were identified on the basis of fifth setiger, prostomium, and pygidium morphology, gill distribution, eyespots, colour and size. Descriptions of species encountered in the research are given in Rainer 1973, Read 1975, Blake and Kudenov 1978. Presumptive identifications of *B. knoxi* and *P. hoplura* were confirmed by Dr. Rainer who first described *B. knoxi* (Rainer1973). Initial identification of *Boccardia proboscidea* Hartman was made by Dr. Geoff Read (National Institute of Water and Atmospheric Research, New Zealand). Non-spionid polychaetes found on or within the shell were not speciated but were included in total polychaete counts where appropriate. Calcareous tube-building polychaetes, such as spirorbids and *Pomatoceros* sp. (Figure 2.4), both usually grouped in the family Serpulidae (Fauchald, 1977), were not included in polychaete counts.

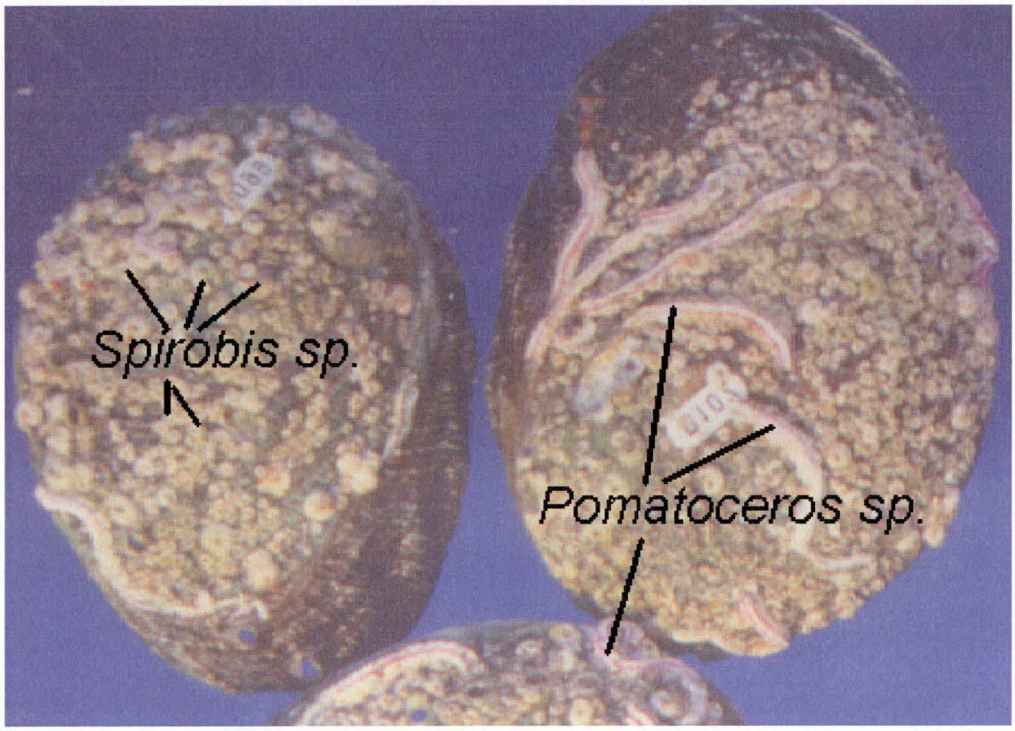


Figure 2.4 Calcareous tube building polychaetes fouling abalone shells

Figure 2.5 *Boccardia knoxi* worms inside characteristic chimney tubes

2.5 Boccardia knoxi chimneys

Preliminary investigation of mud worm infestation showed that *B. knoxi* had a distinctive transparent tube or chimney at the burrow entrance (Figure 2.5). Such chimneys were typically 2-10 mm in length and were produced approximately a month after *B. knoxi* settlement.

While other Tasmanian mud worm species at times produced a burrow entrance structure, the *B. knoxi* chimney was considered distinctive enough to be a useful diagnostic tool. Chimneys survived the death of the worm by many months in the field and for at least eight months in a laboratory test. Remnant, heavily infested abalone from mortality episodes in 1997 were found to have a 90% occupancy rate of live *B. knoxi* on the basis of chimney counts (mean *B. knoxi* count 36.5, SD=19, n=40 shells). This fell to 55% for shells of abalone dead for several months, with a mean live *B. knoxi* count of 23 (SD=15.5, n=40). Thus, chimney counts allowed potential estimation of present and/or past *B. knoxi* numbers. Severe shell fouling interfered with accurate assessment of chimneys. Caceres- Martinez (1999) also found that spionid polychaetes survived the death of the molluscan host.

2.6 Method for expulsion and quantification of spionids

Mud worm infestation levels were quantified using chemical vermifuges to expel polychaetes from shell burrows or the surface of the shell. A mixture of 0-dichlorobenzene and phenol were added to seawater to give a final concentration of 100 PPM 0-dichlorobenzene and 500 PPM phenol based on the methods of Mackenzie and Shearer (1959) and Handley (1997). Abalone were shucked and shells placed in either 50 ml or 400 ml screw lid sample pots depending on size. Pots were at least 60% full (v/v) of vermifuge solution. When mud worm colonisation was considered relatively recent, shells were immersed in the vermifuge solution in the morning and removed in the late afternoon. Longer time periods tended to kill small polychaetes and post larvae rendering identification difficult. Shells with obvious signs of mud worm infestation were exposed to the

vermifuge solution overnight at a minimum temperature of 15° C. After vermifuge exposure the contents of pots were drained through 90 μm sieves and worms were rinsed to petri dishes for examination with a dissecting microscope. In addition, the surface of the abalone shell was examined using a dissecting microscope (10x magnification) to include any polychaetes only partly expelled from burrows. On occasion mud worm blisters were dissected to check for the presence of polychaete eggs and retained worms.

2.7 Estimation of spionid kill efficacy

The distinctive *B. knoxi* chimney allowed an estimate of treatment efficacy by comparison of chimney counts to worm numbers post treatment. This was termed the estimated individual percentage (EI%) kill and calculated:

$$\text{EI\%Kill} = \frac{\text{No. chimneys - No. live } \textit{B. knoxi}}{\text{No. chimneys}} \bullet 100$$

Comparison of surviving worms of other species was calculated by comparison of treatment and control group means. This is referred to as the group mean comparison (GMC) percentage kill. Kill data for *B. knoxi* was calculated by the latter method when excessive fouling rendered chimney counts difficult and when untreated control group data indicated that more than approximately 30% of *B. knoxi* burrows no longer contained live worms.

2.8 Growth

Length of abalone was measured to the nearest 0.1 mm using callipers and, after towelling dry, weight was measured to the nearest 0.1 g. When abalone were tagged, soft plastic tags (Hallprint, South Australia) were fixed to dried shells with fast drying adhesive, allowing mean individual growth to be calculated. Specific Growth Rate (SGR) which gives a measure of growth independent of abalone size

was calculated as the difference between the natural logarithms of the initial and final measures (in mm or g), divided by time and multiplied by 100.

2.9 Blister Assessment

The extent of mud worm blistering was assessed by two methods: a subjective score and an estimate of the percentage of shell with blisters (blister cover). The subjective score was based on approximate area of blister damage, depth, and degree of apparent shell healing. Scores of 0,1,2 and 3 were possible and the system referred to as the subjective shell damage rating (SSDR). Scores of "0" indicated an absence of shell blistering. Scores of "1" indicated light damage, typically blistering to less than 10% of the shell area with blisters characteristically flat and yellow or alternatively well healed with a thick coating of shell nacre over them. An SSDR of "2" indicated damage to 10-25% of the shell area with blister characteristics like that of rating "1" near the upper end of the affected area range, or alternatively raised brown to black blisters over a lesser area of the shell. Shells with raised brown to black blisters occupying at least 25-30% of shell area often with deformity around the apex were assigned the maximum SSDR of "3" This rating was based on shells observed in the original mortality episodes that lead to the research. Examples of shell damage ratings are given in Figure 2.6.

Assessment of percentage blister coverage was made by tracing blisters and shell perimeter on flexible plastic with a printed grid. Areas of blisters and total shell were then calculated by counting squares and percentage blister coverage calculated. Blister coverage was further assessed by classification into "active" and "healed" blisters (Figure 2. 6C). Healed blisters were described as those blisters with a substantial amount of shell nacre deposited over them and which in time could become virtually indistinguishable from the normal shell. Active blisters included those displaying the yellow colour of early conchiolin deposition through to brown and black blisters as a result of the "mud" deposits showing through a relatively thin

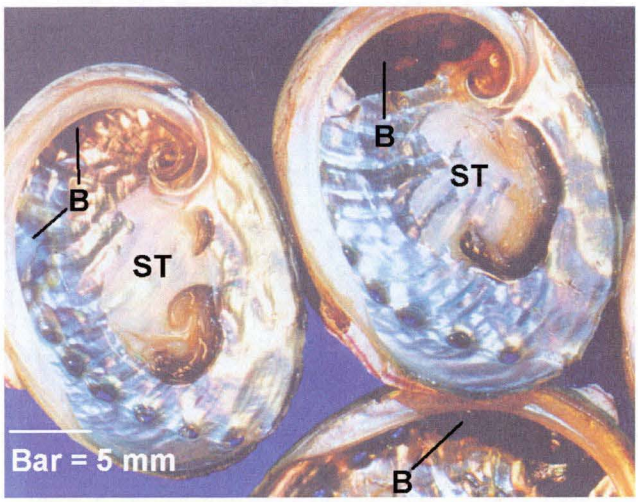


Figure 2.6A SSDR "1", B = Blisters, ST= soft tissue remnant

Figure 2. 6B Three shells with SSDR of "2"

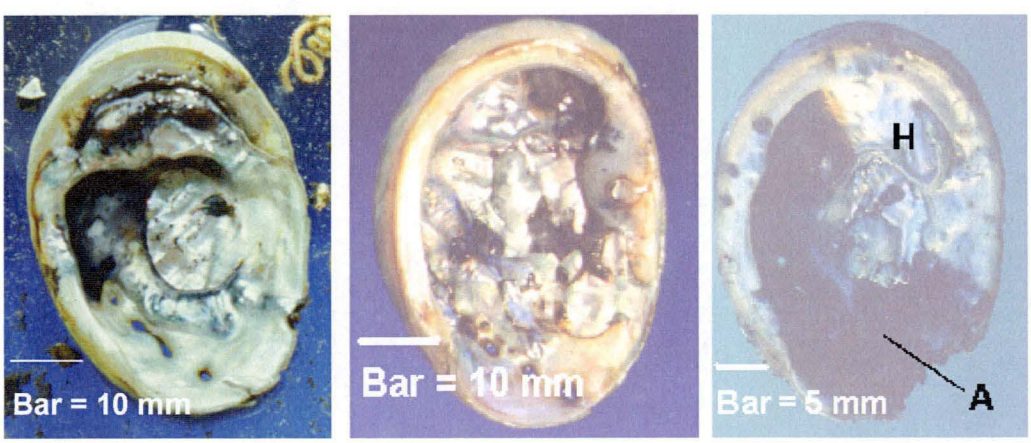


Figure 2.6C Three shells with SSDR of "3", A = Active blister, H = Healed Blister

surface layer. This distinction between blister types was intended to help quantify the age of blisters and the host response to spionid infestation.

2.10 Clinical Pathology

Haemolymph samples were drawn from one or both of two locations. In the first, based on the method of Harris (1999) a small cube of flesh was removed from the foot with a scalpel blade, severing haemocyte channels, and approximately 1.0 ml of haemolymph/tissue fluid transferred from the wound with a syringe. The second bleed site was the cephalic sinus (Jorgensen et al. 1984), located 2-3 mm deep on the midline between the mouth and the anterior portion of the foot. Samples were taken using a 1 ml syringe and 27 gauge needle.

Preliminary sampling showed that it was rarely possible to obtain sufficient sample volume from the cephalic sinus in abalone less than 50 mm in length. Therefore, although this bleed site may be preferable in causing less tissue damage it was not used routinely as many experimental animals were too small. Comparisons between the two bleed sites are presented in Chapters 5 and 8.

Fluid drawn from the appropriate sample site was centrifuged at 1000 *g* for 3 minutes to remove haemocytes. Samples not analysed immediately were frozen at -20°C for subsequent analysis. Measurement of protein, glucose and all ions except copper was performed on a Roche Cobas-MIRA automatic analyser. Samples were diluted 1:4 for sodium, potassium, chloride and calcium and 1:40 for magnesium. Sodium and potassium were measured using ion-selective electrodes. Chloride was assayed spectrophotometrically using the thiocyanate method (Cobas-MIRA, 1987). Calcium and magnesium ion concentration was determined spectrophotometrically using the arsenazo method (Cobas-MIRA, 1987). Glucose and protein were undiluted and determined spectrophotometrically using the hexokinase and biuret methods, respectively (Cobas-MIRA, 1987). Copper concentration was determined by adding 0.1 ml sample to 1 ml of 20% trichloroacetic acid and reading at 324.8 nm on a spectrophotometer.

2.11 Haemocyte counts

Total haemocyte counts were performed with a haemocytometer (Assistent, Germany) on samples taken from one or both of the sites described in section 2.10. Approximately 0.2 ml of haemolymph/tissue fluid was transferred from the wound to the haemocytometer with a syringe. Counts were performed at ×200 magnification by light microscopy within minutes after bleeding to avoid aggregation of haemocytes. Five of 25 small squares were counted on each side of the haemocytometer and a mean calculated for each sample. Count data were converted to cells.ml^{-1} by multiplying by 50 000.

2.12 Histology

Abalone soft parts were fixed in 10% seawater formalin. Routine processing was performed using a Shandon Hypercenter XP for dehydration through ethanol to xylene (Lamberg and Rothstein 1978). This was followed by paraffin embedding on a Tissue tek work centre and sectioning on a Microm HM 340 microtone at 4 μm. Routine Harris's Haemotoxylin and Eosin (H & E) staining was performed using a Leica Jung Autostainer XL for automatic staining. Sections were mounted in DPX and examined and photographed through a light microscope.

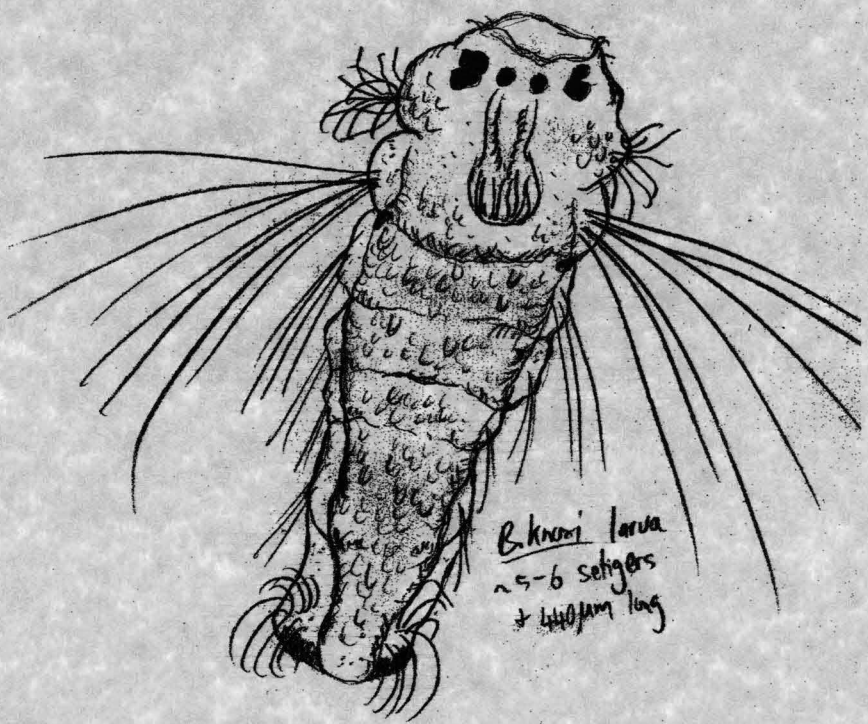
2.13 Statistical Methods

Statistical analysis was conducted using Genstat 5 software (©1998 Lawes Agricultural Trust, Rothamsted Experimental Station). For temporal change experiments data were subjected to one or two way ANOVA with time, and time and treatment regime as factors, respectively. Assumptions of normality and homogeneity of variance were checked by examination of the residual plots option in Genstat. REML (Residual Maximum Likelihood technique) was used instead of ANOVA for unbalanced data sets (where sample numbers varied between times). Where individual data sets were transformed this is noted in the methods section of

the appropriate chapter. Separation of means was performed by the use of the LSD (Least Significant Difference) function in Genstat.

Spionid count data when the values were low tended to be non- normally distributed and the non-parametric Mann-Whitney U Test, suitable for such data, (Sokal and Rohlf, 1995) was used. Non-parametric methods were also used for analysis of subjective SSDR ordinal data.

The non-parametric or distribution free Kruskall-Wallis test (Zar, 1984) was preferred to ANOVA when data sets were small and the nature of the underlying distribution was unknown. Mean separation was performed by the method of Zar (1984) relating to this test.


B. knoxi larva
~5-6 setigers
+ whatever long

Frontispiece: Boccardia knoxi larva

Chapter 3
REPRODUCTION AND SETTLEMENT OF SPIONIDS

3.1 Introduction

This chapter is concerned with the natural history of spionids infesting cultured abalone, with a focus on reproduction, larval dispersal and settlement. Basic understanding of such factors is necessary to develop management plans that seek to minimise the impact of *B. knoxi* and other mud worms.

There is a significant body of work on reproduction and larval development of spionids, including Wilson 1928, Hopkins 1958, Dorsett 1961, Blake 1969 and Anger et al., 1986. Two basic modes of spionid reproduction have been described (Woodwick 1977, Radashevsky 1994). One mode involves the production of large numbers of relatively small eggs. Each egg develops into a larva and leaves the maternal burrow at a size of <500 μm (with between 3-6 setigers or body segments) and swims actively, feeding in the plankton. Such larvae are referred to as planktotrophic and species with this strategy have been termed "broadcasters" (Skeel 1979). The alternative reproductive strategy termed lecithotrophy involves the release of larvae that feed on yolk supplies, non-fertilised ("nurse" eggs), or small larvae (< 500 μm) within the burrow. By this strategy relatively small numbers of large larvae are produced (Woodwick 1977, Radashevsky 1994). These larvae have limited swimming ability and spend little if any time in the plankton. Larvae of both developmental patterns metamorphose and settle at about the 14-18 setiger stage when they are approximately 1.3 to 1.8 mm in length.

In New Zealand, *B. knoxi* has been previously studied by Read (1975) and Handley (1997, 2000). The former author found that reproduction occurred throughout the year, mainly through lecithotrophy, but that an uncommon planktonic stage was also possible. By contrast, Handley (1997, 2000) found evidence of only planktotrophic larval development during the spring.

Discovering the reproductive strategy, including its timing, for Tasmanian *B. knoxi* may determine when risk to stock is greatest and allow development of avoidance strategies. Examination of larval dispersal patterns may assist in determining the risk infested stock pose to abalone subsequently transferred to farm sites. Acquisition of such knowledge is an important step in formulation of spionid management plans.

3.2 Methods

3.2.1 Descriptive methods, including larval rearing and spionid reproductive histology

Mud worm egg strings were dissected from blisters in cultured abalone and examined using Olympus dissecting and compound microscopes with camera adaptors. Where required, specimens were relaxed with 10% magnesium chloride.

Spionids were cultured by releasing larvae from capsules at approximately the 5-setiger stage and rearing in 1 l plastic aquaria. Water was filtered to 10 μm and larvae were supplied with cultured microalgae (School of Aquaculture, University of Tasmania – Launceston) including *Isochyrsis galbana*, *Pavlova lutheri* and *Tetraselmis* sp. Ambient water temperature was 13-16 °C and larvae were captured on 90 μm sieves every 3-4 d and relocated to clean aquaria. For reproductive studies presumptive adult (>10 mm) *B. knoxi* were relaxed in 10% magnesium chloride, preserved in 10% seawater formalin and processed for routine paraffin histology (Chapter 2). Worm sections were for the presence of eggs at 100x. Eggs were measured with an eye piece graticule and the presence of mature eggs, up to 95 μm in diameter (Read, 1975) was recorded.

3.2.2 Timing of spionid settlement

To investigate the null hypothesis that spionid settlement occurs year round, duplicate groups of uninfested stock from farm 1 were transferred to the sea-based study sites at Aquatas and the Huon Aquaculture Company (Chapter 2) monthly: August-January 1998/99, June- December 1999 and July-December 2000. Generally, 10 of these previously uninfested abalone were sampled per replicate the month following transfer with subsequent sampling every 2-4 months. Additional, known infested stocks present at farms prior to August 1998 were also examined periodically. Abalone were housed in tube or basket type rearing vessels and maintained as described in Chapter 2.

An additional sea-based study site became available in 1999 when Tasmanian Scallops P/L commenced abalone farming on the east coast (Chapter 2). A limited amount of sampling was performed, taking advantage of normal commercial operations. Uninfested abalone from Farm1 (Chapter 2) had been transferred to the Tasmanian Scallops lease in January and February 1999 and 2000. This circumstance was used to determine the timing of subsequent spionid settlement through sampling (n=10-20) in April 1999 and six additional times between April and November 2000.

Potentially infested samples from the three field study sites were relocated to the Fish Health Laboratory, Launceston in seawater and mud worms were detached from shells using the chemical vermifuge method (Chapter 2). Examination of shells, from abalone field-exposed for one month showed that spionids present were < 3mm in length and, thus, worms of this size present in shells with prior mud worm exposure were also considered to provide evidence of recent larval settlement. Mud worm blisters were investigated for the presence of adult worms and their reproductive state assessed. Spionids were considered to have mature eggs within the body if these ruptured easily and were released during examination. The presence of extruded egg strings was recorded and they were examined for the presence of active larvae within the capsules. As not all samples contained blisters within a given month the absence of body egg and extruded shell egg/larvae data in

results Tables 3.1 and 3.2 does not confirm the absence of these stages of reproductive development.

3.2.3 Larval dispersal experiment

In this experiment the null hypothesis that abalone at variable distances from a source of potential infestation would be at equal risk of spionid colonisation was tested. Abalone infested with *B. knoxi* and *P. hoplura* since August 1998 were used as a source of infestation. Fifty infested animals were placed in each of 2 baskets; 1 m from each side of the centre of a 50 m longline orientated north-south at the Aquatas lease. Uninfested stock from farm 1 (Chapter 2) were transferred to the source of infestation baskets and to further baskets located at 5, 10 and 25 m intervals on each side of the centre (Figure 3.1). Additionally, two further baskets each containing uninfested stock were hung directly 1 m below the source of infestation baskets (Figure 3.1). It was necessary to hang these additional baskets below the infestation source baskets, rather than next to them, to avoid entanglement. Two size classes of clean abalone were used to indicate spionid settlement. Mean lengths of the size classes were 58.5 mm (SD=5.3 mm, n=127) and 37.2 mm (SD=4.9 mm, n=360). The larger animals were the last mud worm-free abalone of the size available and, thus, smaller abalone were used to minimise the risk of low settlement compromising the experiment. Sixteen large and 90 small size class animals were placed in each basket at the standard depth (Figure 3.1), including the two source of infestation baskets. The additional baskets hung 1 m under the infestation source contained only small class size clean abalone. The experiment commenced May 1999 and spionid infestation was quantified in February 2000 after presumed spring/summer spionid reproduction.

Spionid counts were made in relation to distance from source of infestation. The standard vermifuge method was used to expel worms from shells (Chapter 2) and each shell was examined for the presence of characteristic *B. knoxi* chimneys (Chapter 2). Large size class abalone were analysed individually but small size class abalone were examined in replicates of five since the latter were found to be minimally infested. Statistical analysis was by ANOVA after square root transformation of data.

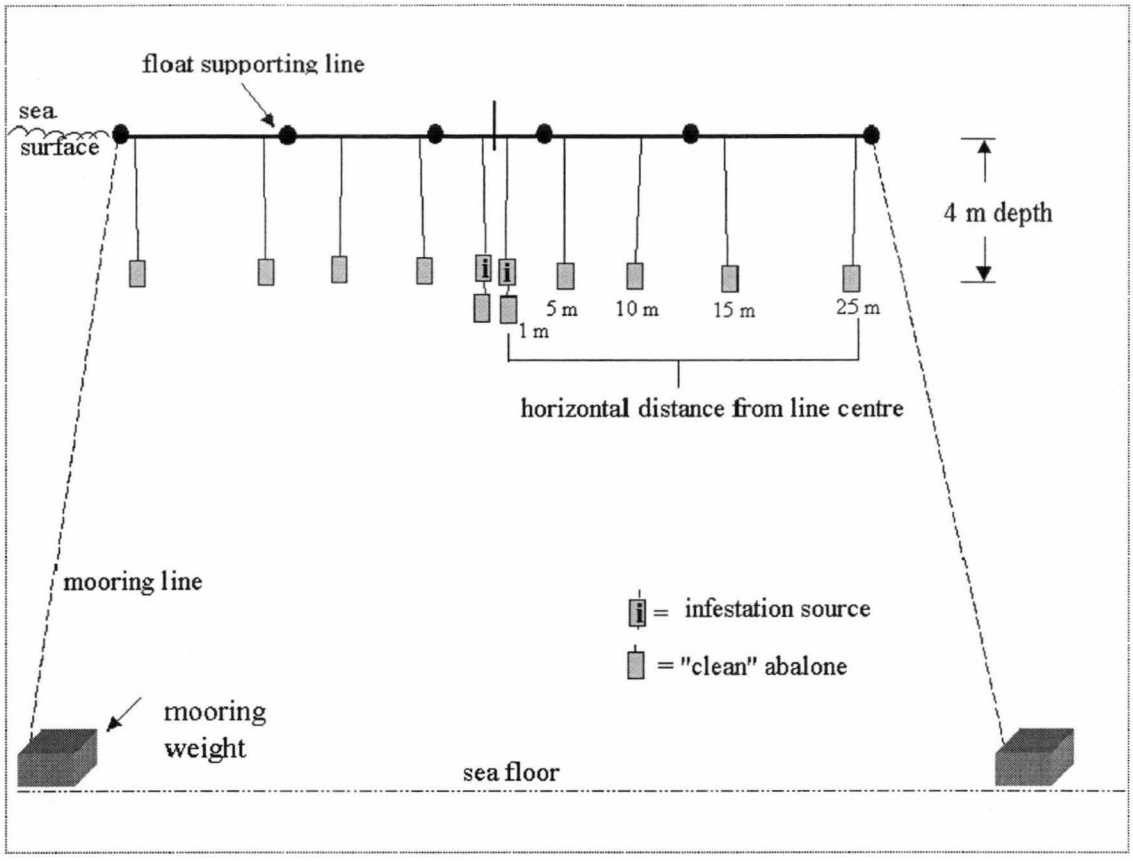


Figure 3.1 Experimental design for larval dispersal experiment

3.3 Results

3.3.1 Descriptive studies

Most mature specimens of *B. knoxi* were 10-20 mm in length with the occasional specimen up to 35 mm recorded. The mean length of a sample of fixed specimens was 16.3 mm (SD=16.1 mm, n=15) with a mean setiger count of 98.8 (SD=95.5, n=13). Specimens of *P. hoplura* were generally 20-30 mm in length with larger specimens of 50 mm (>200 setigers) recorded. For line drawings of these species and technical descriptions refer to Blake and Kudenov (1978), Rainer (1973) and Read (1975). Images of adult spionids are given in Figure 3.2. The developmental sequence for *B. knoxi* including egg string, planktonic larvae and larvae approaching settlement are given in Figures 3.3-3.8. The comparable sequence for *P. hoplura* is shown in Figures 3.9-3.11. Egg strings of *B. knoxi* had a mean of 21.8 capsules per string (SD=8.6, n=9) and capsules contained a mean of 32.8 larvae (SD=8.7,n=46) for a mean brood yield of 715 larvae. Egg strings often exhibited a greenish tinge (Figure 3.3) and all eggs developed into larvae, there was no evidence of nurse eggs. Asetigerous embryos were about 240 μm long, growing to approximately 350 μm at 5 setigers when they were capable of surviving outside the capsule (Figure 3.6). Swimming larvae of this size were strongly phototaxic and their translucent bodies had a green tinge. Larvae released from egg capsules at a mean size of 369 μm and cultured reached 670 μm 5 d later and 900-1200 μm (16-17 setigers) about 2 weeks after release. By this time larger larvae were commencing metamorphosis with the beginning of crawling behaviour, and showed the presence of 5th setiger spines and palps (Figure 3.8). A small number of larvae were maintained for a further week after this but failed to grow any larger.

Histology of adult *B. knoxi* showed that parapodial cavities were densely packed with oocytes of 90-110 μm in diameter in May (Figure 3.12). Sections of worms sampled June to August showed essentially empty body cavities (Figure 3.13) – corresponding with extrusion of eggs from the body to the shell burrow. Immature oocytes were seen developing in the ovary by November/December

(Figures 3.14 and 3.15) and by January to March had been released once more into the parapodial cavities to mature (Figure 3.16).

Egg strings of *P. hoplura* tended to be deep yellow in colour (Figure 3.9) and were often 20-30 mm in length with > 30 capsules. Nurse eggs were always observed and capsules generally contained 40-50 such eggs and 4-6 larvae of approximately 500 μm early in the development sequence (Figure 3.10). Larvae as large as 1900 μm were observed within capsules near the exhaustion of nurse egg supplies. Small worms 2000-2500 μm were sometimes observed excavating burrows adjoining the maternal burrow apparently having never left the host shell. No direct evidence of planktotrophic larval development of *P. hoplura* was observed in infested abalone.

Another species of spionid, *Boccardia proboscidea* Hartman, was seen infrequently allowing some assessment of its reproductive cycle. Egg strings, with distinctive oval capsules (Figure 3.17) characteristic of the species (Woodwick, 1977; Blake and Kudenov, 1981) were seen once in each of December 1998 and September 1999. Lecithotrophic larvae 1.4 mm long were observed within capsules October 1999. Adults with well-developed eggs within their bodies were seen between September 1998 and January 1999.

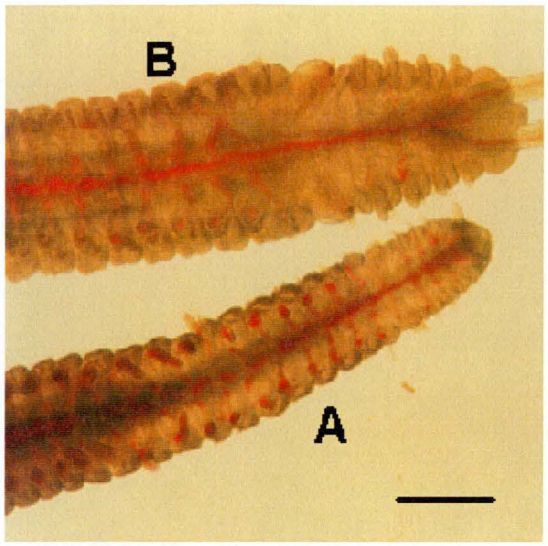


Figure 3.2 Anterior ends of *B. knoxi* (A) and *P. hoplura* (B), palps missing or obscured. Bar = 1 mm.

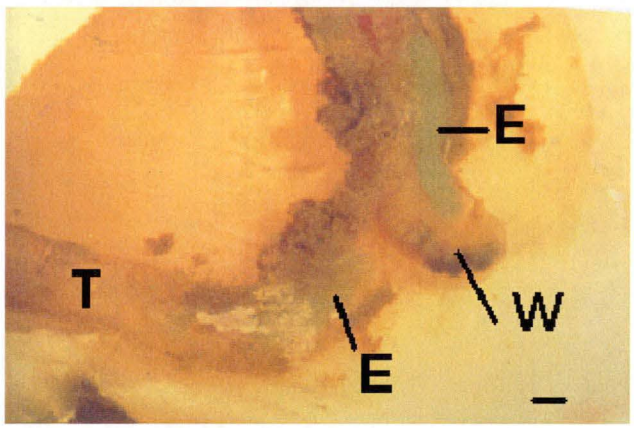


Figure 3.3 *B. knoxi* egg string: T = worm tube, W = worm, E = egg string. Bar = 1 mm.

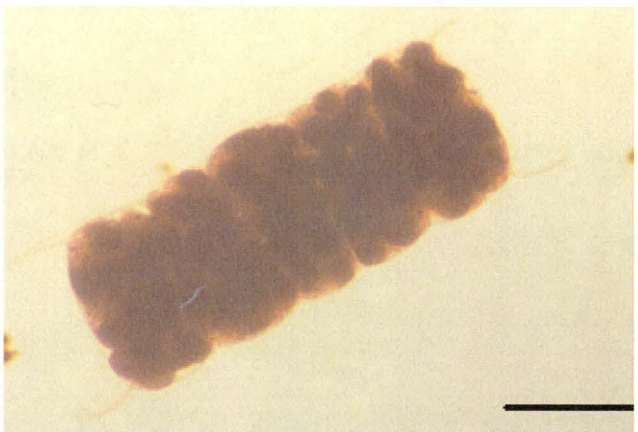


Figure 3.4 Five capsules from *B. knoxi* egg string. Bar = 0.5 mm.

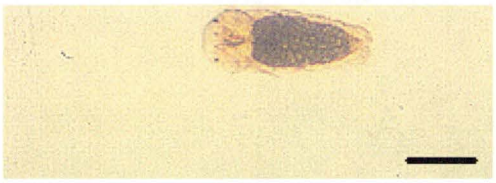


Figure 3.5 *B. knoxi* larva broken out of capsule. Bar = 100 μm.

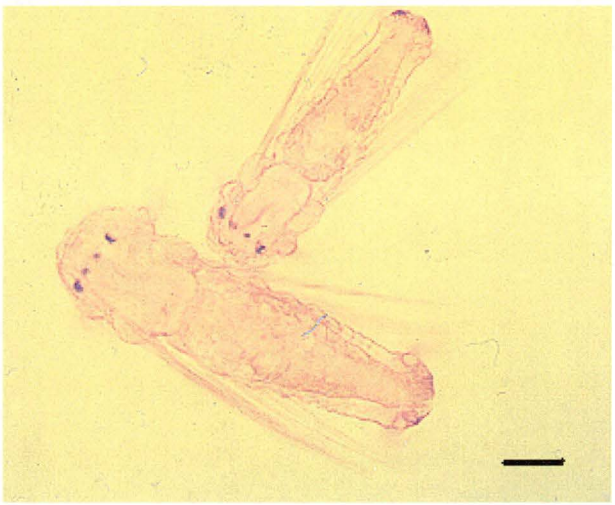


Figure 3.6 *B. knoxi* larvae 5-6 setigers. Bar = 100 μm

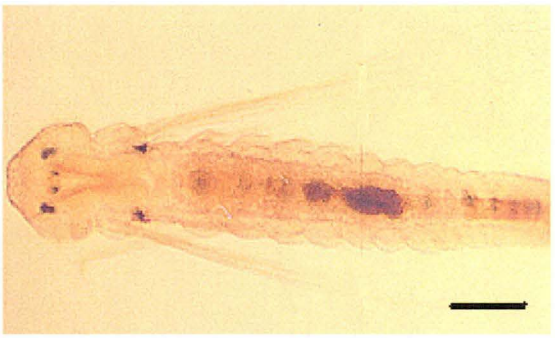


Figure 3.7 *B. knoxi* larva 14-15 setigers. Bar = 100 μm.

Figure 3.8 Crawling *B. knoxi* larva at 17-18 setigers and 1200 μm. Bar = 200 μm.

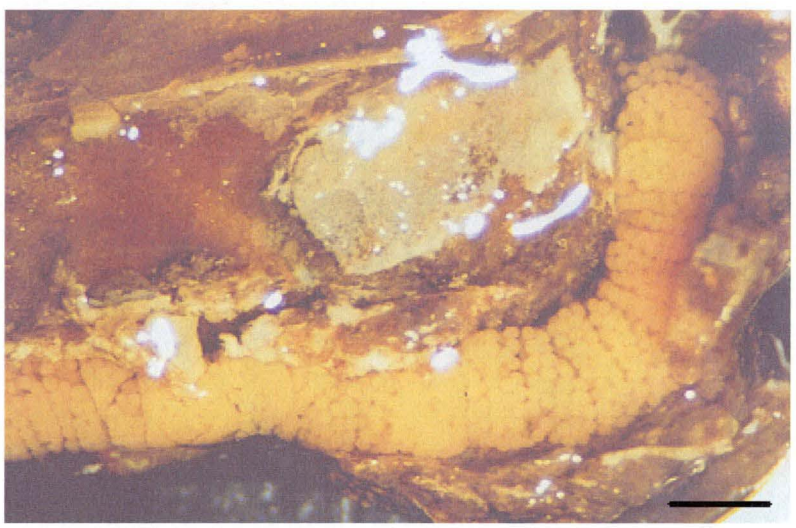


Figure 3.9 *P. hoplura* egg string, bar = 1 mm

Figure 3.10 *P. hoplura* larvae and nurse eggs removed from capsule, bar = 100 μm.

Figure 3.11 Cultured *P. hoplura* larva, 1200 µm, 18 setigers. S = 5 th setiger spines. Bar = 100 µm.

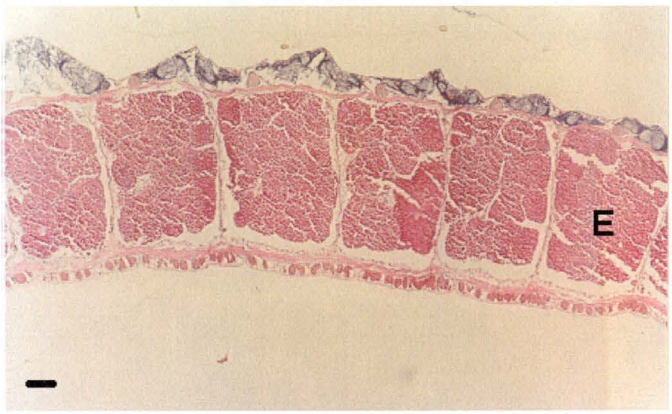


Figure 3.12 Section through mature *B. knoxi*. E = egg masses, Bar = 100 µm.

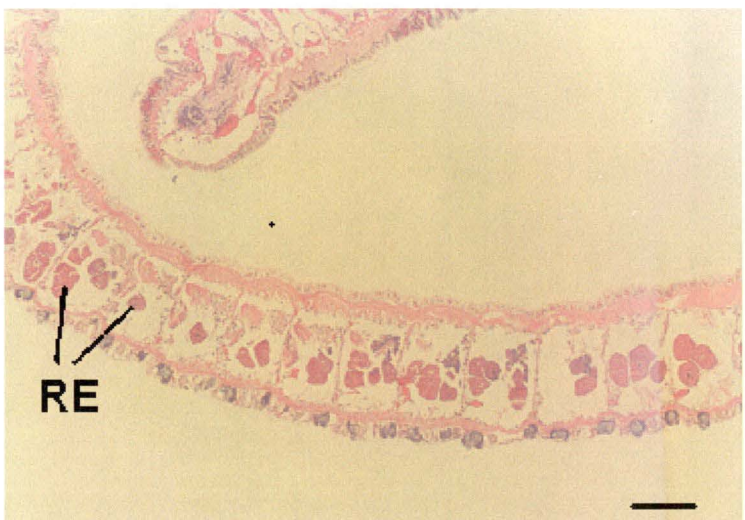


Figure 3.13 Section through *B. knoxi* post extrusion of Eggs. RE = remnant eggs, bar = 400 µm.

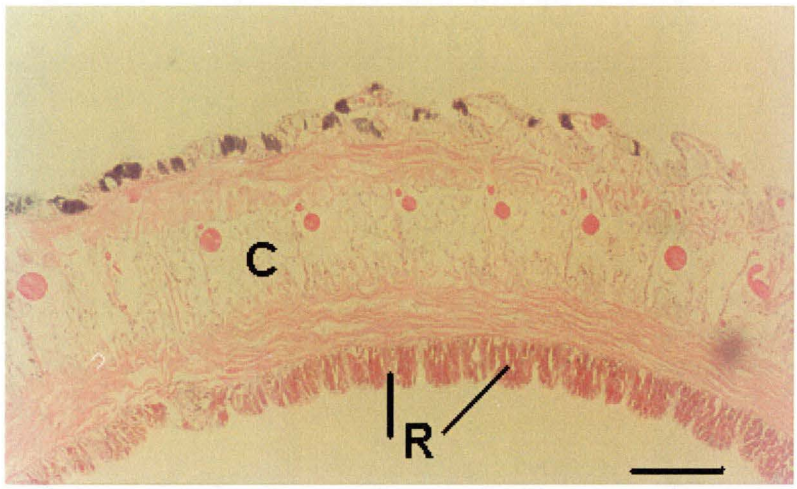


Figure 3.14 Section through *B. knoxi* showing re-developing eggs (R) yet to migrate to the coelom (C). Bar = 400 μm.

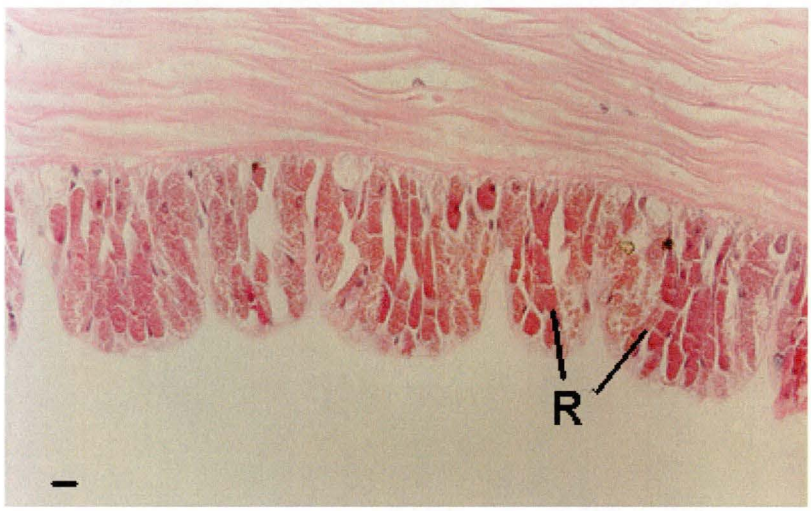


Figure 3.15 Close-up of re-developing eggs (R). Bar = 10 μm.

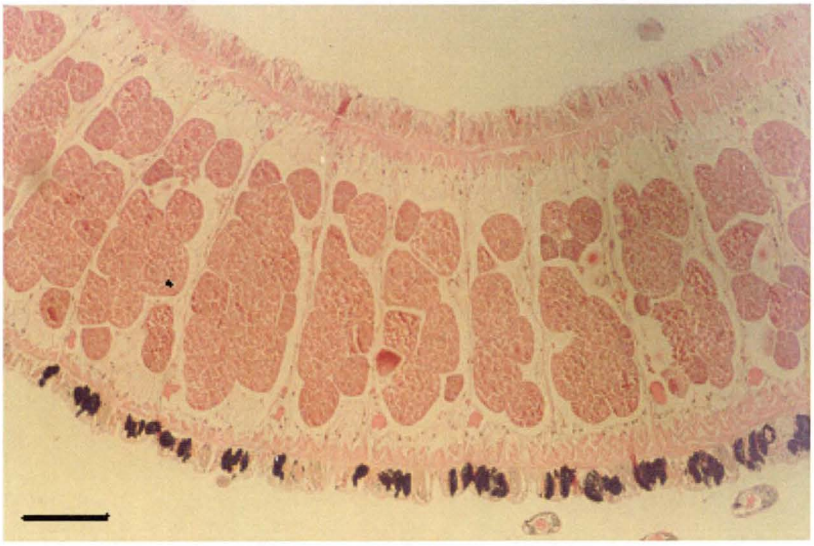


Figure 3.16 Section through female *B. knoxi* approaching ripeness. Bar = 400 μm.

Figure 3.17 Egg capsule of *B. proboscidea* showing pigmented larvae within. Bar = 100 μm.

3.3.2 Timing of settlement

Settlement of *B. knoxi* larvae was confined to the months of the Austral spring in the period 1998-2000 at Aquatas and Huon Aquaculture (Tables 3.1 and 3.2). Mature body eggs were observed between April and August and extruded eggs were seen in June and September. Viable unhatched larvae were present in most August samples at both study sites with the earliest observation June 1999 and the latest in January 2001 (Tables 3.1 and 3.2).

Post-larvae and adult (with mature body eggs) *P. hoplura* were observed in every calendar month at one time or another during the study period (Tables 3.1 and 3.2). As evidenced by the cohabitation of post-larvae and adults in burrows, the majority of post larval worms were considered to be the lecithotrophic offspring of worms already present in blisters. At times, post-larval *P. hoplura* were found inhabiting otherwise spionid-free shells and it was considered possible such specimens had undergone planktonic development. Most such specimens were found in the spring and summer (Tables 3.1 and 3.2).

Spionids were rarely seen in samples from the east coast site. A settlement of P. *hoplura* was observed in October 1999. No *B. knoxi* post larvae were recorded but one sub adult (5-10 mm) worm was present in each of August and October 2000 samples, suggestive of a winter settlement.

Table 3.1.Presence-Absence of *B. knoxi* and *P. hoplura* life history stages at Aquatas

	Sep 98	Oct 98	Nov 98	Dec 98	Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99
Mature body eggs										+•	•	•
Extruded egg strings										+•		•
Unhatched larvae												+
Settled post-larvae	+•	+•	+•		•• #		•• #				•	•

+ = *B. knoxi*; • = *P. hoplura*; •# = suspected planktonic *P. hoplura* post larvae

	Sep 99	Oct 99	Nov 99	Dec 99	Jan 00	Feb 00	Mar 00	Apr 00	May 00	Jun 00	Jul 00	Aug 00
Mature body eggs	•		•		•		•	•		•		
Extruded egg strings	•		•				•		•	•		
Unhatched larvae	•	+	•									
Settled post-larvae	+•	+•	+•• #		•		•• #	•		•		

	Sep 00	Oct 00	Nov 00	Dec 00
Mature body eggs	•			
Extruded egg strings	•			
Unhatched larvae				
Settled post-larvae	+•• #	+•	+•	•• #

Table 3.2 Presence-Absence of *B. knoxi* and *P. hoplura* life history stages at Huon Aquaculture

	Sep 98	Oct 98	Nov 98	Dec 98	Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99
Mature body eggs		•		•		•		+•	•		•	•
Extruded egg strings	+	•									•	•
Unhatched larvae										+		+•
Settled post-larvae		+•• #	+•		•• #	•	•• #	•	•	+•• #		•

+ = *B. knoxi*; • = *P. hoplura*; •# = suspected planktonic *P. hoplura* post larvae

	Sep 99	Oct 99	Nov 99	Dec 99	Jan 00	Feb 00	Mar 00	Apr 00	May 00	Jun 00	Jul 00	Aug 00
Mature body eggs	•			•	•		•				•	+•
Extruded egg strings	•	•	•	•	•		•					•
Unhatched larvae	+•	+•		•	•		•					+
Settled post-larvae	+•• #	+•• #	+•• #	•• #	•	•	•• #					•

	Sep 00	Oct 00	Nov 00	Dec 00	Jan 01
Mature body eggs			•		
Extruded egg strings			•		
Unhatched larvae			•		+**
Settled post-larvae		+•	+•		•• #

+** = record consists of 2 worms

3.3.3 Larval dispersal

Spionid infestation was low and variable. There were approximately less than three worms per replicate of five abalone for smaller stock and less than three worms per abalone for larger animals (Figures 3.18 and 3.19). On the smaller size abalone significant differences in settlement existed between baskets on the long line for *B. knoxi* counts ($F=4.08$, df 8 126, $P<0.001$) and *P. hoplura* counts ($F=2.29$, df 8 126, $P<0.05$) but not for *B. knoxi* chimneys ($F=1.84$, df 8 127, $P>0.05$). Data for the larger abalone showed significant differences between baskets for *B. knoxi* chimneys ($F=4.19$, df 6 99, $P<0.001$), *B. knoxi* worm counts ($F=4.33$, df 6 99, $P<0.001$) and *P. hoplura* ($F=3.23$, df 6 96, $P<0.01$).

There were no significant differences for any measure of spionid settlement between "clean" abalone housed in the infestation source baskets located 1 m north and south of the long line centre (Figures 3.18 and 3.19). Nor were there any differences in infestation rates between abalone in the 25 m south location and stocks in these centre baskets. However, abalone located in the 25 m north baskets were generally significantly less infested than stocks on the opposite end of the long line and in the centre (Figures 3.18 and 3.19). For simplicity, the baskets housing small abalone only and hung a metre under the central baskets are not shown in Figure 3.18. There was no significant difference ($P>0.05$) for *B. knoxi* chimney counts between the source, and those baskets hung 1 m below the source, for either replicate. Data for *P. hoplura* were more variable showing a significant difference ($P<0.05$) for one of two replicates.

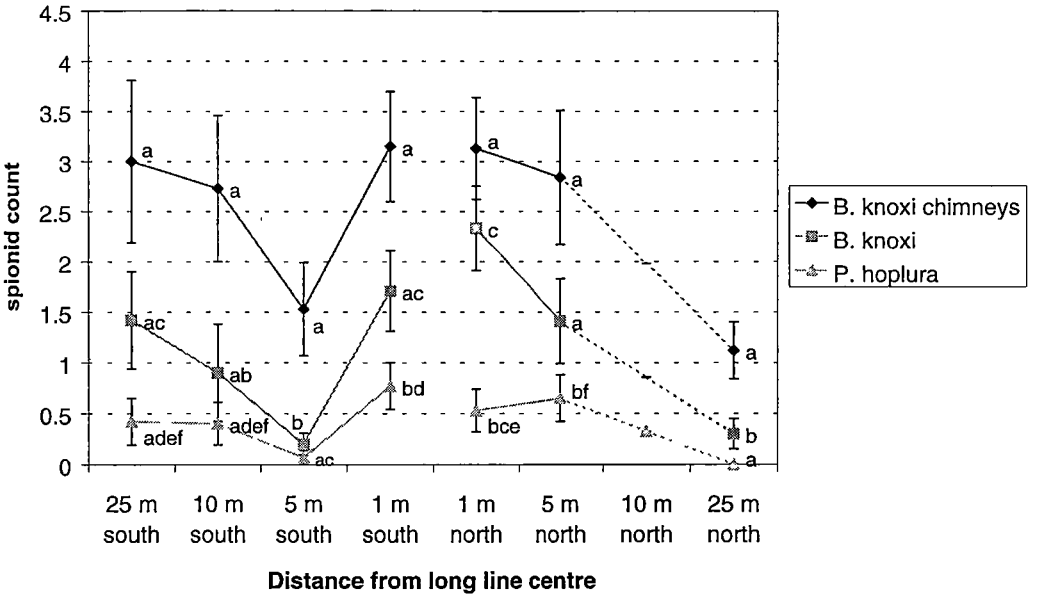


Figure 3.18 Spionid settlement of 40 mm abalone at variable distance from central source of potential infestation. Spionid counts: means of replicates of 5 ± SE. Data series with shared superscripts are not significantly different (P>0.05).

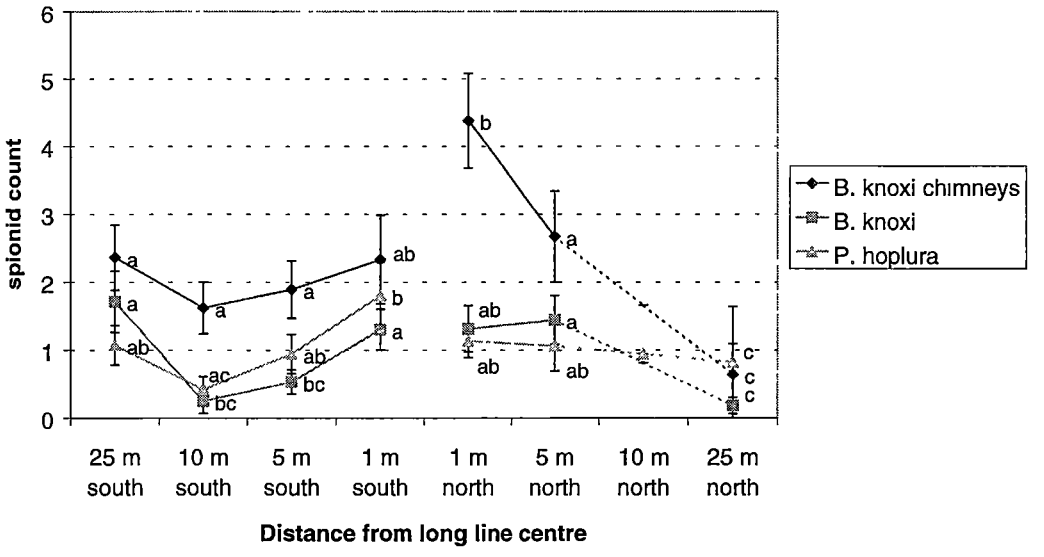


Figure 3.19 Spionid settlement of 60 mm abalone at variable distance from central source of potential infestation. Mean spionid count ± SE. Data series with shared superscripts not significantly different (P>0.05).

3.4 Discussion

The spionid species *B. knoxi* appears to be somewhat larger in Tasmanian abalone than described in New Zealand (Rainer 1973, Read 1975). There was no evidence of brooded *B. knoxi* production as reported by Read (1975). Rather, the lack of nurse eggs, release of larvae from capsules at the 4-5 setiger stage and relatively large clutch size indicate planktonic larval production as described by Handley (1997,2000). The estimated clutch size of > 700 larvae for Tasmanian *B. knoxi* is similar to that of other spionids with planktotrophic development including *P. websteri* (500-550, Blake 1969), *Boccardia chilensis* Blake and Woodwick (460±160, Skeel 1979), *P. ciliata* (225-400, Wilson 1928; 200-1100 Dorsett 1961) and *Polydora haswelli* Blake and Kudenov (1200-2400, Skeel 1979).

By contrast, *P. hoplura*, although physically larger than *B. knoxi*, was less fecund consistent with the observed lecithotrophic development. Maximum fecundity was less than 150-180 larvae, consistent with estimates for the species by Skeel (1979), Radashevsky (1994), and Gromadzki (1994). There was no direct evidence of planktonic larval production within *P. hoplura* blisters in cultured abalone. However, the observed presence of *P. hoplura* larvae in otherwise spionid free stock at some distance from the nearest source of infestation may suggest a dispersal ability not normally associated with large lecithotrophic larvae. Possibly, such larvae could be transported on debris or seaweed. Anecdotal evidence from abalone farms that feed seaweed to stock suggests that some spionid larvae can be transported by this means – and freshwater soaking is employed to minimise risk of such infestation. At the Aquatas site, however, there was no direct evidence of drift seaweed accumulating on rearing vessels.

It would not be without precedent if *P. hoplura* were capable of producing two types of larvae to maximise its reproductive potential. Wilson (1928) noted that if larval *P. hoplura* within capsules containing brood eggs were removed and fed on micro-algae they developed normally and were indistinguishable from larvae allowed to complete lecithotrophic development. As seen previously *B. knoxi* apparently produces both brooded and planktonic larvae in New Zealand.

Skeel (1979) notes that *B. chilensis* produces brooded larvae in Tasmania but planktonic larvae in New South Wales. Gromadzki (1994) found that *P. haswelli* in South Australia could produce both planktonic and brooded larvae in differing proportions in the same geographic location. Likewise, Woodwick (1977) discusses characteristics of planktonic and lecithotrophic *B. proboscidea* larvae from the same population.

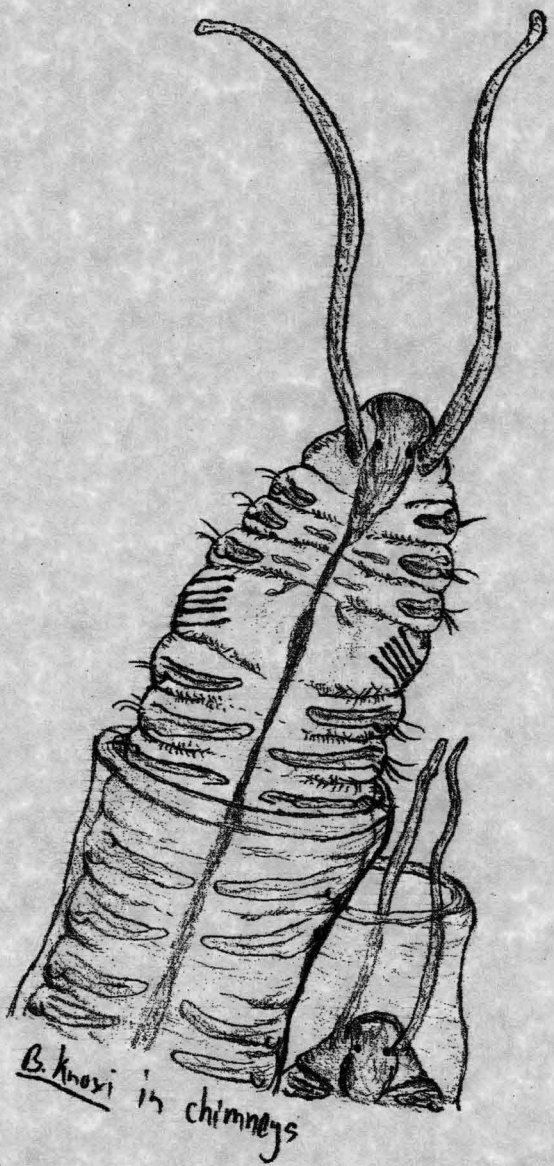
The ability of *P. hoplura* to produce lecithotrophic larvae at most times of the year once established in the abalone may render control difficult. Fortunately the presence of *P. hoplura* post-larvae on clean stock was mainly confined to spring and summer in the south of the state (Tables 3.1 and 3.2) and east coast. Likewise, *B. knoxi* settlement data, and other observations of the reproductive cycle (including histology) strongly suggest that if stock are placed after November they will remain substantially spionid free until the following spring.

Results from the distance dispersal experiment were of little use in elucidating what degree of risk infested stock pose to clean stock. There was differential spionid settlement between baskets but not in a pattern consistent with greater infestation close to a source of spionids and tapering off at a distance. Greater spionid settlement rates would probably be required to demonstrate any relationships between infestation sources, distance and the environment.

Deduction based on observed modes of reproduction suggests that once established, numbers of *P. hoplura* worms in a host will increase due to maternal care and limited larval dispersal ability. Larvae of *B. knoxi*, by contrast, leave the host at an early stage and survive at least 2 weeks in the plankton. Thus, by the time *B. knoxi* larvae are competent to settle they may be potentially removed at some distance from the original source. The extent of *B. knoxi* colonisation seen here is approximately an order of magnitude less than that seen in mud worm infested stock examined in 1997 (Chapter 1) suggesting *B. knoxi* larval prevalence and /or survival may vary considerably year to year.

3.5 Conclusion

B knoxi was shown to have an exclusively planktonic mode of larval production with a limited dispersal period occurring consistently in the spring 1998-2000. This suggests infestation can be avoided by placement of stock post November allowing up to 9 months growth before potential infestation the following spring. A second species, *P. hoplura* was found to be capable of year reproduction once established. However, dispersal data suggested much initial infestation could be avoided by post December site transfers. Infestation rates for *B. knoxi* were low compared to available previous data suggesting recruitment rates may vary considerably year to year.


B. knoxi in chimneys

Frontispiece: Boccardia knoxi worms inside chimneys

Chapter 4
CHEMICAL IMMERSION TREATMENT OF SPIONIDS

4.1 Introduction

The previous chapter focused on avoidance and minimisation of spionid infestation. In this, the first of two chapters on treatment options, means of treating spionid infestation by the use of chemotherapeutic agents are explored. An effective treatment option would be an important tool in an overall plan to minimise the economic impact of spionid infestation.

Because of the economic importance of oyster cultivation worldwide treatment options have been investigated (Whitelegge 1890, Korringa 1952, Mackenzie and Shearer 1959, Bailey-Brock and Ringwood 1982, Nel et al., 1996). By contrast, there is little literature on the incidence and effects of spionids in cultured abalone and consequently no prior studies on treatment options. Effective treatment agents will need to be safe for abalone and farm personnel, be cost effective and capable of registration for use in Australian culture facilities.

This chapter examines the efficacy of a range of chemotherapeutants from the major vermicidal drug classes used in terrestrial agriculture and also antiparasitics used in aquaculture industries. Representatives of the former group tested include mebendazole, fenbendazole, levamisole, ivermectin, trichlorofon, febantel and pyrantel embonate. Chemotherapeutants with a history of use in aquaculture included: potassium permanganate, methylene blue, metronidazole, dimetronidazole, praziquantel, malachite green and formalin. These agents may be effective against protozoa, fungi and some metazoan ectoparasites (refer to review by Cross and Needham, 1988). In addition the vermicidal properties of hydrogen peroxide and gentian violet were investigated. Freshwater soaking previously used for spionid treatment in oyster species (Whitelegge 1890, Tonkin 1997) was also assessed for suitability in treating infested abalone.

4.2 Methods and Materials

4.2.1 Experimental animals and rationale

When these experiments commenced fewer than 1000 heavily infested abalone remained from the original mid-1990's spionid outbreak. These were made available for treatment research and continued to die during 1998 as experiments proceeded. Consequently, initial experimental emphasis was on performance of screening trials to identify promising treatments while experimental animals remained. Later it was found that infested shells retained viable mudworms for months after death of abalone (Chapter 2) and such "empty shells" were used in many experiments. As infested abalone shells of any type became scarce further experiments were conducted *in vitro* using spionid mudworms, primarily *B. knoxi*, extracted from infested Pacific oysters. Experiments on toxicity of potential treatment agents to abalone were conducted on uninfested healthy stock when suitable infested stock were unavailable. Follow up work for the most promising treatment was conducted on a limited supply of lightly infested stock considered still curable.

4.2.2 *In vitro* trials of toxicity to spionids

Spionids were sourced from known *B. knoxi* infested Pacific oysters resident at the Huon Aquaculture Company. Shells containing presumptive *B. knoxi* were determined by the presence of the distinctive transparent "chimney" (Chapter 2). The majority of spionids selected for use were *B. knoxi* but there was no attempt to exclude worms of other species from closely related genera.

Mud worms were removed from shells by mechanical destruction with bone forceps or by the use of chemical vermifuges (Chapter 2). Where chemical extraction was used harvested worms were placed in several changes of fresh seawater then left for 1-2 d in aerated seawater. Worms were sorted using a dissecting microscope to select substantially intact specimens with normal colour.

During toxicity trials mud worms were housed in 200 ml specimen jars for initial exposure to treatment chemicals and the duration of the recovery period. No aeration was supplied and water changes were performed daily. Toxicity trial recovery periods were up to 3 weeks and worms were not fed. Spionids were examined grossly at water changes and microscopically once or twice per week. Specimens were considered dead if no movement could be detected and decomposition was observed. Generally, chemical treatments were tested at three or more different concentrations with one specimen jar housing five to ten worms used for each concentration. Exposure times were 3 h unless otherwise stated. Ambient temperature ranged from 15-18 °C.

4.2.3 Spionids *in situ* toxicity trials

Abalone used in these trials were remnant severely *B. knoxi* infested animals from Huon Aquaculture. On-going stock deaths indicated the health of experimental animals was severely compromised. Abalone were 40 - 80 mm in length and 4-5 years old. In excess of 10 distinctive *B. knoxi* chimneys were present on individuals selected for therapeutant trials. Experiments used live infested abalone, infested "empty shells" or a combination of both as indicated.

Potential treatments were generally tested at 3 different levels of chemical concentration or exposure times with an untreated control. Ten individually tagged abalone were used per treatment level. Plastic tags (Hallprint, South Australia) were adhered using "supa glue" (Selleys, Padstow, NSW). During treatment abalone and "empty shells" were removed to 5 l aquaria outside the recirculating system (Chapter 2); recovery occurred in the recirculating system after chemical exposure and thorough rinsing. Aeration was supplied during experimental exposure and afterwards in the holding tank. Exposure times were generally 3 h and water temperature was 15-18 °C. Abalone and shells were maintained post treatment for approximately 1 week. This allowed sufficient time for mud worms and abalone to recover from treatment if they were capable of doing so. It also allowed sufficient time for dead mud worms to decompose, assisting in the assessment of surviving

worms. Abalone mortality was recorded and dead animals were removed, shucked and the shells replaced in the aquaria. At the termination of the recovery period surviving mud worms were driven from their shells by chemical vermifuges (Chapter 2) and quantified.

Treatment efficacy was estimated by comparison of group means between control and treatment groups (Chapter 2). Alternatively, where appropriate, the individual kill rate for each shell was calculated by use of the EI%Kill (Chapter 2).

4.2.4 Abalone Toxicity Trials

Live, infested abalone were not always available for *in situ* efficacy testing of potential mud worm treatments. The health of heavily infested animals was poor. Thus, healthy abalone were used for toxicity work on promising chemical treatments. This stock was sourced from farm 1 (Chapter 1). Most of the abalone ranged in size from 40-50 mm and 12-25 g and were 2-3 years old. A second population of blacklip abalone (18-20 mm, 0.7-1.1 g and10-11 months of age) was also used on occasion. Toxicity trials were conducted in the aquaria described previously. Generally 5-10 abalone were used for each treatment level tested. A reserve population of at least 20 abalone were housed in aquaria within the recirculating system to serve as a control. Immersion exposure times were 3 h at 14-16 ° C unless otherwise stated. In retrospect, it would have been preferable to also remove the control aquaria for the appropriate exposure time. After experimental exposure abalone were returned to the recirculating system until any mortality had apparently ceased. Where no mortality occurred animals were maintained for 1-3 weeks with formulated commercial abalone feed provided.

4.2.5 Selection of treatment agents

The research aim was to test a wide range of potential treatment agents while infested stock remained. Literature on concentration rates for particular chemotherapeutants was used as a starting point as summarised in Table 4.1, along with concentration ranges and exposure times for chemical agents used in the present study. Additionally, freshwater immersion as previously described by Korringa (1952) and Bailey-Brock and Ringwood (1982) for treatment of spionid infested oysters was tested. The effect of a combination of drugs present in dog worming tablets (Exelpet®, "All-Wormer for Dogs", Wyong, NSW, 2259) was also examined. In this way, febantel, pyrantel embonate, and praziquantel were tested at 25, 14.4 and 5 $mg.l^{-1}$ respectively, through to 5 times these concentrations.

Following the completion of this research a further reference on the use of ivermectin against salmon louse in Atlantic salmon was brought to the author's attention. Johnson and Margolis (1993) found that doses of 0.05 $mg.kg^{-1}$ administered in feed reduced the infestation.

Table 4.1 Concentration and exposure times for chemotherapeutic agents used in this and previous studies.

Chemical agent	Previous studies			This study
	Concentration & time	Parasite or Disease agent	Reference	Concentration & time
Potassium	10 PPM 30-60 min	Ectoparasites,	1	2-50 $mg.l^{-1}$ 3-4 h
permanganate	25 PPM 15 min	Fungi	1	
Malachite green	2 PPM 30-60 min	Fungi & protozoa	1	1-20 $mg.l^{-1}$ 3 h
	10 $mg.l^{-1}$	Bacteria	2	
Trichlorofon	300 PPM 15-60 min	Salmon lice	3	0.1-1000 $mg.l^{-1}$ 3 h
	0.5 $mg.l^{-1}$	Flukes, *Argulus* sp.,	4	
		Copepods		
Praziquantel	10 $mg.l^{-1}$ 3 h	Flukes	1,4	10-100 $mg.l^{-1}$ 3 h
		Tapeworms	5	
Formalin	167-250 PPM 1 h	External parasites	1	5-200 PPM
	25-100 PPM 4 h	Protozoa	2	
Metronidazole	25 $mg.l^{-1}$	External protozoa	1	5-200 $mg.l^{-1}$ 3 h
Methylene blue	3 PPM	Protozoa & fungi	1	1- 200 $mg.l^{-1}$ 3 h
Gentian violet				5-100 $mg.l^{-1}$ 3-4 h
Mebendazole				50-500 $mg.l^{-1}$ 3 h
Fenbendazole				50-500 $mg.l^{-1}$ 3 h
Levamisole				0.32-640 $mg.l^{-1}$ 3 h
Hydrogen peroxide				50-1000 PPM 3 h
Ivermectin				0.004-0.4 $mg.l^{-1}$ 3 h
Dimetronidazole				20-500 $mg.l^{-1}$ 3 h

Reference Key: [1]Cross & Needham 1988, [2]Owens et al., 1988, [3]Brandal & Egidius 1979, [4]Langdon 1990, [5]Lester 1988

4.2.6 Follow up experiments performed on lightly infested stock

Further experiments using lightly infested stock still considered treatable, were performed to assess treatment options showing the most promise from the experiments conducted *in vitro*, *in situ* with severely infested stock, and from abalone toxicity trials. Experimental animals were chosen from a small pool of lightly infested, *B. knoxi*-positive, 40-50 mm abalone on the basis of visible chimney structures. Animals were individually tagged as described previously.

A chemotherapeutic bath experiment consisted of four treatment groups, including an untreated control, each with two replicates of 10 abalone. The treatments consisted of immersions in one of the three following chemicals for 3.5 h at 15 °C: gentian violet 5 $mg.l^{-1}$, mebendazole 200 $mg.l^{-1}$ or potassium permanganate 15 $mg.l^{-1}$. The untreated control group was maintained in 5 l aquaria within the recirculating system at 15 °C. More properly, this group should have been removed from the recirculating system for the 3.5 h exposure period.

Following treatment abalone were maintained in the recirculating system for 1 week at 15°C without food and shells were processed as described previously to quantify polychaete infestation. Data were analysed using chi-square analysis (Zar 1984). Chi-square analysis was performed by comparing the number of presumed dead and live worms recovered at the end of the trial. Dead worms were calculated as number of chimneys minus number of live worms recovered for each shell

4.3 Results

4.3.1 Screening trials

Major findings for the considerable body of screening trial data are described below; more detail is presented in Appendix 4 as indicated. Fresh water immersion of spionids *in vitro* proved 100% fatal after 10 min exposure. Exposure of spionids *in situ* proved ineffective for 1 h immersion, rising to 40% GMC (Group Mean Comparison) kill at 2 h, at the cost of 80% abalone mortality (Appendix 4A).

Potassium permanganate exposure appeared to have some potential against spionids *in vitro* at 15-20 $mg.l^{-1}$ and 100% mortality was seen 7 d post exposure at 50 $mg.l^{-1}$. Potential for abalone mortality was seen at $\geq$ 20 $mg.l^{-1}$ with 80% mortality seen 16 d post treatment at 20 $mg.l^{-1}$, 4 h immersion. Three hour treatment of spionids *in situ* at 25 $mg.l^{-1}$ $KMnO_4$ resulted in 60% abalone mortality and 11% GMC spionid kill (Appendix 4B).

Gentian violet exposure was found to be 100% lethal to mud worms *in vitro* at 10 $mg.l^{-1}$ 4 d post treatment. Some abalone mortality was seen at 10 and 20 $mg.l^{-1}$ but not at 5 $mg.l^{-1}$, 17 d post treatment. Four hour exposure of mudworms *in situ* to 7.5 $mg.l^{-1}$ gentian violet resulted in 23% GMC kill (Appendix 4C).

Spionid mortality *in vitro* approached 100% 9-10 d post treatment with mebendazole and fenbendazole in the range 50-500 $mg.l^{-1}$. No abalone mortality was seen following 50 $mg.l^{-1}$ exposure to mebendazole for 9 h or 200 $mg.l^{-1}$ exposure for 3 h. Lethality to spionids *in situ* was estimated at 53% by GMC kill. Similarly, no fenbendazole toxicity to abalone was seen at 250 $mg.l^{-1}$, and GMC kill was 41% for spionids tested *in situ* empty shells (refer to Appendices 4D and 4E)

Levamisole immersion (64 and 640 $mg.l^{-1}$) of spionids *in situ* resulted in GMC kills of 42 and 66%, respectively, and resulted in abalone mortality of 50 and 100%, respectively. Follow up abalone toxicity trials using healthy 20 mm stock showed no mortality 8 d post exposure to 64 $mg.l^{-1}$ levamisole, rising to 33% at 320 $mg.l^{-1}$ and 100% mortality at 500 $mg.l^{-1}$. Larger healthy stock (40-50 mm) showed no mortality at 320 $mg.l^{-1}$ 18 d post exposure (Appendix 4F).

Malachite green exposure of mud worms *in vitro* was 100% lethal at 5 and 10 $mg.l^{-1}$ but ineffective at 1 $mg.l^{-1}$. Concurrent exposure to spionids *in situ* (empty shells) gave GMC kill of 38 and 60% at 5 and 10 $mg.l^{-1}$, respectively. Toxicity trials on healthy abalone (20 mm) resulted in 10% mortality at 5 $mg.l^{-1}$ and approached 100% at 10 $mg.l^{-1}$ (Appendix 4G).

Spionids challenged *in vitro* showed limited mortality 8 d post exposure to trichlorofon at concentrations of 500 and 1000 $mg.l^{-1}$. Healthy 40-50 mm abalone were not killed by exposures of up to 500 $mg.l^{-1}$ 11 d post treatment (Appendix 4H). Similarly, praziquantel ($\leq$ 100 $mg.l^{-1}$) produced limited mortality to spionids *in vitro* 20 d post treatment (Appendix 4I). Hydrogen peroxide in the range 200-1000 PPM was lethal to 60% of spionids *in vitro*. No abalone mortality was seen at in healthy stock at 200 PPM (Appendix 4J).

Formalin produced 30% mortality at 100 PPM for spionids *in vitro*, dropping to 22% (GMC kill) for spionids *in situ* empty shells. At 200 PPM *in vitro* spionid mortality exceeded 80%, dropping to 49% for concurrent estimate *in situ*. Follow up trials on healthy abalone showed no mortality a week post treatment at 100 PPM but 90% mortality at 200 PPM (Appendix 4K).

No mud worm toxicity was observed *in situ* using 0.004 and 0.04 $mg.l^{-1}$ ivermectin; however, 57% (GMC kill) was observed at 0.4 $mg.l^{-1}$ ivermectin. Mortality of 85% for infested abalone was observed at 0.4 $mg.l^{-1}$. Follow up experiments using 0.1-0.3 $mg.l^{-1}$ showed ivermectin was ineffective against spionids *in vitro* and 100% lethal to healthy 20 mm abalone above 0.2 $mg.l^{-1}$ (Appendix 4L).

A commercial product, Exelpet®, "All-Wormer for Dogs" containing febantel, pyrantel embonate and praziquantel (at 125, 72 and 25 $mg.l^{-1}$, respectively, in solution) was ineffective against mud worm *in vitro* and non lethal to healthy abalone at twice this concentration (Appendix 4M).

Metronidazole at 200 $mg.l^{-1}$ was non-lethal to spionids *in vitro* and, similarly, dimetronidazole produced limited mortality at 500 $mg.l^{-1}$ (Appendix 4N). Methylene blue was non toxic to spionids *in situ* (using empty shells) and to healthy abalone at up to 10 $mg.l^{-1}$. Further trials using spionids *in vitro* showed no mortality at up to 200 $mg.l^{-1}$ (Appendix 4O).

4.3.2 Follow up trial on lightly spionid infested abalone

Gentian violet at 5 $mg.l^{-1}$, mebendazole at 200 $mg.l^{-1}$ and potassium permanganate at 15 $mg.l^{-1}$ were chosen on the basis of screening trial data for follow up *in situ* bath trials on lightly spionid-infested abalone. Spionid killing efficacy data were of marginal statistical significance (P=0.057, Pearson Chi-square value 7.5, 3 df). Mebendazole was the most effective treatment for *B. knoxi* by bath exposure, which resulted in a mean estimated individual percentage kill of 32.5% (Table 4.2). Gentian violet treatment resulted in the death of 1 in 20 experimental animals.

Table 4.2 Treatment of lightly infested abalone by one of three chemical baths (means ± SD).

	Control	gentian violet 5 $mg.l^{-1}$	mebendazole 200 $mg.l^{-1}$	potassium permanganate 15 $mg.l^{-1}$
mean *B. knoxi* chimneys	1.5 ± 0.9	1.5 ± 0.9	1.6 ± 0.8	1.6 ± 0.8
mean surviving *B. knoxi*	1.4 ± 0.7	1.1 ± 0.6	1.1 ± 0.8	1.3 ± 0.6
mean EI% *B. knoxi* Kill	3.8 ±11.9	18.8 ± 32.5	32.5 ± 43.0	13.5 ± 26.5
Abalone Mortality (%)	0	5	0	0

n=20 each treatment

4.4 Discussion

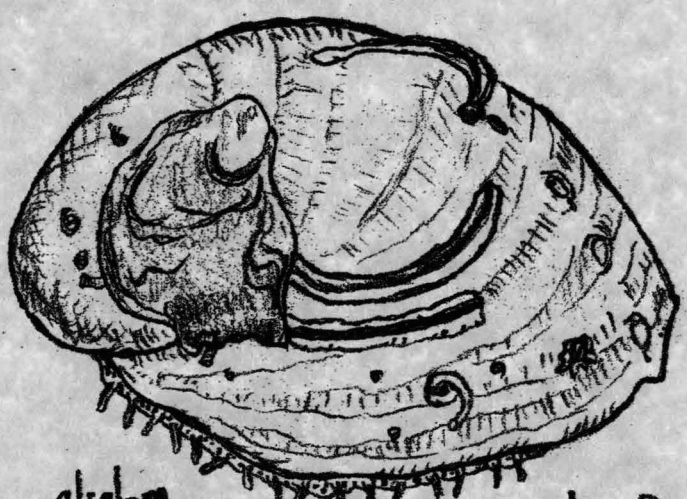
Sixteen potential chemotherapeutic agents and fresh water immersion were tested. Available literature on effective concentrations for bath treatment of fish and shellfish parasites was used as a starting point. It was concluded that trichlorofon, praziquantel, hydrogen peroxide, febantel, pyrantel embonate, metronidazole, dimetronidazole and methylene blue were not effective against spionid polychaetes at high doses relative to available literature.

Immersion in freshwater, potassium permanganate, gentian violet, levamisole, malachite green, formalin and ivermectin was highly effective against mudworms *in vitro*. However, the protection conferred by the burrows of spionids *in situ* rendered effective concentrations unsafe for abalone. The benzimidazoles: mebendazole and fenbendazole showed the largest differential between the toxic level to mud worms *in vitro* (100% mortality at 50 $mg.l^{-1}$) and toxic level to stock (0% mortality at 200 $mg.l^{-1}$ or greater). However, as for other chemotherapeutic agents, the protection conferred by spionid burrows rendered the drugs largely ineffective for bath treatment. This may be compounded by their poor solubility in water.

The benzimidazole class of drugs may have some potential for microencapsulation as treatment for various polychaetes, (including families other than the Spionidae such as Serpulids and Sabellids), infesting abalone. This approach has been explored previously by Shields et al. (1997) using copper sulphate as the effective agent to treat a sabellid polychaete. More recently Overweter (2000) used microencapsulated albendazole with success against the calcareous tube building polychaete *Galeolaria* sp.

4.5 Conclusion

Immersion treatment of mud worms infesting abalone was rendered ineffective by lack of penetration into burrows and insufficient toxicity differential between spionids and abalone. Further work in this area was not considered a high priority because an alternative approach, set out in the next chapter, appeared more promising.



abalone c/ shell fouling - oyster, Pomatoceros, spirorbids + barnacle.

Frontispiece: abalone with shell fouling

Chapter 5
AIR DRYING TREATMENT AND ITS AFFECTS ON ABALONE

5.1 Efficacy of air drying as a mud worm treatment

5.1.1 Introduction

This section explores the viability of air drying as a treatment for spionid infestation of abalone. Rouse (2000) states that polychaetes are virtually incapable of resisting desiccation and, thus, this approach seeks to exploit any potential tolerance to drying between parasite and host. Regular air exposure is a feature of inter-tidal oyster farming and a major benefit of this farming method is minimisation of mud worm infestation (Skeel 1979, Smith 1984). Air drying of oysters has also been used as a specific treatment for mud worms (Whitelegge 1890, Smith 1984, Nell and Smith 1988). The application of this method for treatment of abalone may be regarded as somewhat novel given that abalone are not cultured inter-tidally and are often subtidal in distribution. The apparent distribution may be misleading, however, since abalone are most vulnerable to human fishing pressure in the inter-tidal zone. The author has observed wild *H. rubra* and *H. laevigata* exposed by the tide in isolated parts of the state and similar observations have been reported for *H. rufescens* in California (Tjeerdema et al.,1991). Another species, *H. cracherodii* has an intertidal to shallow subtidal distribution in California and Mexico (Friedman 2002, pers. Comm.) Shipment out of water is also the standard way on transporting abalone species to market. Thus, some abalone species have at least a limited capacity for survival out of water.

The distribution of the *B. knoxi* in Pacific oysters in Tasmania is almost exclusively subtidal rather than intertidal. This is also the case in New Zealand. Handley (1997) presents data showing an absence of *B. knoxi* in intertidal oysters studied and the presence of the spionid in subtidal oysters. These observations suggest that there may be potential in the air drying approach, at least in the case of *B. knoxi*.

A treatment option is considered a desirable component of an overall strategy to minimise the impacts of mud worms in susceptible areas of Tasmania. Air drying, if proven as a treatment, would be preferred by industry over the use of a chemical agent.

5.1.2 Methods and Materials

General experimental protocol

Experimental animals were obtained from one of four commercial abalone culture facilities. Where treatment experiments were conducted in the laboratory stock was transported in water to the laboratory from the source farm as were animals treated on farms then assessed in the laboratory.

Treatment group abalone were removed from culture vessels and exposed for air drying on a suitable substrate such as plastic sheet. Where replication was used separate substrates were provided for each group. Temperature was measured at half-hour intervals as was humidity using a wet and dry bulb hygrometer (Masons type, Zeal, London). In some experiments abalone were individually tagged (Chapter 2). Untreated control animals were returned to water as soon as possible after tagging. Replicates were kept in separate mesh bags or plastic aquaria.

Following treatment, air exposed and control abalone were maintained in the recirculating system (Chapter 2) for one week at 15-16°C with out food. This allowed time for dead mud worms to decompose clarifying assessment of surviving mud worms. Abalone were subsequently shucked and shells were placed

individually in vermifuge solution (mixture of 500 PPM phenol and 100 PPM 0-dichlorobenzene) in seawater over night to expel worms from shells (Chapter 2). All spionid mud worms were speciated but other polychaetes were grouped as "others" and may contribute to total polychaete data in some experiments. Fouling polychaetes such as *Pomatoceros* sp. and Spirorbids were excluded from counts. Treatment efficacy data was calculated by the estimated individual percentage kill (EI%Kill, Chapter 2) or by group mean comparison (GMC), as appropriate.

Statistical analysis

Data were analysed by chi-square analysis or Mann-Whitney U Test. Chi-square analysis was performed on some data sets for *B. knoxi* counts by comparing the number of presumed dead, and the count of live worms recovered at the end of the trial. Dead worms were calculated as number of chimneys minus number of live worms recovered for each shell. The Mann-Whitney U test was used for *B. knoxi* when chimney counts were not made and for other species of polychaetes. This distribution-free test was preferred as data sets consisted of worm counts that were generally with low values and consequently were not normally distributed. One-way ANOVA was performed in Trial 1 where large numbers of spionids were present.

Stock history and method specifics

Trial 1. Treatment of remnant severely infested abalone

Severely *B. knoxi* infested blacklip abalone present at Huon Aquaculture since 1994 or 1995 were assigned to treatment groups of 3, 5 or 8 h air exposure. Abalone were 50-70 mm in length with SSDR scores of 2-3 (Chapter 2) and blister coverage to > 25 % of shell area. Ten shells were assigned to each air exposure time, the majority of which were live abalone (Table 5.1). Five shells including one live abalone were used for an untreated control.

Abalone were placed in plastic aquaria and located in light shade outside at the Fish Health Laboratory. Air temperature inside aquaria ranged from 21-24° C during exposure, which was staggered so that all aquaria were returned to water at the same time. The trial was conducted in late November 1997.

Trial 2. Treatment of stock infested with B. knoxi < 6-8 months, four hours at 24 °C

Blacklip abalone approximately 3 years old and positive for *B. knoxi* chimneys were selected from available stocks at farm 1 (Chapter 2). Forty animals were chosen which measured an average 44.6 mm (SD=3.6 mm, n=15). The infestation level was low with generally 1-3 chimneys per abalone. Based on previous stock inspections and usual spionid growth rates, larval settlement was estimated to have occurred about 8 months previously.

Twenty abalone were assigned to each of treatment and control groups and tagged (Chapter 2). The treatment group was exposed to air for 4 h at 24 ± 1 °C using reverse cycle air conditioning. The humidity was subsequently estimated at 46%.

Trial 3. Treatment of B. knoxi infested stock at Variable Temperatures

A total of 105 *B. knoxi*-positive blacklip abalone, mean length 47.1 mm (*SD* = 3.9 mm, n = 40) were obtained from the same tank series as in Trial 2, and were similarly lightly infested. Abalone were individually tagged.

Twenty-one abalone in each of 4 treatment groups were exposed to air for 4 h. Air temperatures were: 15, 18, 21 and 24 °C and were controlled using reverse cycle air conditioning or column heating. Humidity values were: 60, 62, 53 and 71% in ascending temperature order. An untreated control group was maintained in the recirculating system at 15°C.

Trial 4. Treatment of B. knoxi infested abalone at variable exposure times

Eighty *B. knoxi*-positive abalone were selected from the same source as Trials 2 and 3 above and tagged. There were five treatment groups (including an untreated control) each consisting of 2 replicates of 8 abalone. The treatment air exposure times were 1, 2, 3 and 4 h. Air temperature was 21 ± 0.5 °C and humidity was 60%. The experiment was conducted in the laboratory using reverse cycle air conditioning. The untreated control group was maintained in the recirculating system at 15°C. In this trial the recovery time was 3 rather than 7 d before processing.

Trial 5. Air drying of old heavily polychaete infested greenlip abalone

A group of 60- 85 mm greenlip abalone was obtained from Tasmanian Tiger Abalone, Dunalley (Chapter 2). The stock was 5-6 years old and had become spionid infested at least 3 years previously. Previously, the abalone were cultured in a sea-based grow out system but had since been transferred to a land-based system that used flow-through filtered water. The animals were survivors of a group with mortality linked to mud worm infestation commencing 3-4 years previously. The health of the stock was considered poor as some of the animals were under weight for their shell length. The shells were eroded on the dorsal surface and had blisters covering 20-30% of the ventral shell area.

From this pool of stock 20 *B. knoxi*-positive abalone were chosen for the experiment and tagged. There were two experimental groups: an untreated control and a group treated by air exposure. Ten animals were assigned to each group with two replicates of five abalone per group. The treatment conditions were 4 h of air exposure at 21 ± 0.5 °C. Treatment was performed in the laboratory using reverse cycle air conditioning and humidity was 60 %. The untreated control group was maintained in the holding system at 15 °C.

Trial 6. Air drying of recently infested stock under field conditions.

This experiment was performed at Huon Aquaculture on December 9 1998. Blacklip abalone (46.5 mm ± 2.9 mm (mean ± SD, n=40)) were approximately 3 years old when transferred to the farm in August 1998. Settlement of *B. knoxi* and *P. hoplura* spionids was known to have occurred in the interval between placement and treatment. Animals were air-dried for 3.5 h at ambient temperature and humidity of 16-18°C and 49-62%, respectively, during the time period 12:30 h to 16:00 h. Abalone were removed from each of two replicate culture vessels and placed on plastic sheet in direct but weak sun light for 5 min and then in shade for the remaining time. Thirty abalone were assigned to control and treatment groups from each replicate. Stock was not inspected to exclude uninfested abalone. The control group was returned to water as soon as possible after selection. From the pool of stock, 20 treated and 20 control abalone (in 2 replicates of 10) were taken to the laboratory for efficacy analysis. The remainder stayed at the site to provide data on long-term treatment benefits (Chapter 7).

Trial 7. Air drying of heavily fouled, P. hoplura infested stock under field conditions

This trial was performed at Aquatas on January 20 1999. Experimental animals had been transferred to the farm in December 1997 at approximately 2 years of age. Treated stock were 30-50 mm in length and had acquired various degrees of fouling with *Pomatoceros* sp., *Spirorbis* sp. and Pacific oysters. Previous examination of the stock indicated *P. hoplura* was the most common spionid present.

Exposure time was 4 h between 10:30 h and 14:30 h. Treatment group animals were placed in direct sunlight for the first 10 min of air exposure and in shade on plastic sheet for the remaining time. Air temperature ranged between 17.5 and 22 °C and air humidity was between 43 and 65%. Twenty animals were assigned at random to control and treatment groups without inspection to exclude

any abalone not infested. The control group was returned to water as soon as possible after selection.

Trial 8. Two and half hour drying of recently B. knoxi infested stock

Blacklip abalone, mean length 42.3 mm (SE =0.6 mm, n=80), were collected from Huon Aquaculture and treated on site 12 November 1999. Stock had been on site for 12-14 months but the infestation rate from the preceding spring 1998 *B. knoxi* settlement period was low. Stock was treated towards the end of the 1999 *B. knoxi* settlement period. The maximum recent *B. knoxi* infestation period was approximately 3 months.

Forty animals were assigned at random to control and treatment groups with out inspection to exclude uninfested stock. The treatment group was placed on a plastic sheet outside in the shade. The day was sunny and air temperature ranged from 16 to 17 °C during the 2.5 h exposure. Humidity was 50 to 54%.

Trial 9 Drying of 14 month mud worm infested blacklip abalone.

Blacklip abalone, mean length 52.6 mm (SE = 1.0 mm, n=20), were collected from Huon Aquaculture and treated outside at the laboratory 25 October 1999. Stock was approximately 4 years old and had been on site for 14 months, acquiring shell fouling in addition to mud worm infestation.

Ten abalone from each of two rearing vessels were assigned to a treatment or control group giving two replicates of five animals per treatment. Control group animals were tagged and returned to water as soon as possible. Treatment group abalone were exposed to air for 3.5 h. Air temperature ranged from 16 to 20 °C (but was generally in the range 16 – 17 °C); humidity ranged from 48 to 59%. Post-treatment abalone mortality data was not collected as animals were further used to collect clinical pathology data (section 5.2, this Chapter).

Trial 10. Repeat drying of abalone previously treated one year earlier

Blacklip previously treated in December 1998 were re-treated December 13 1999. Stock of average length 60.9 mm (SE = 0.8, n = 19) were drawn from the same population as in Trial 6 above. Abalone were dried at Huon Aquaculture for 4 h. Temperature ranged from 15-18°C and humidity ranged from 46 and 58% after an initial reading of 80%.

5.1.3 Results

Trial 1. Treatment of remnant severely infested abalone.

Analysis of variance (arcsine transformed data) of estimated % kill data was highly significant (F=19.2, df 3,34 P<0.001) with a mean 90% kill rate seen for the 8 h exposure. Abalone mortality occurred at 5 and 8 h exposure times (Table 5.1).

Trial 2. Treatment of stock infested with B. knoxi < 6-8 months, four hours at 24 °C

Treatment caused considerable reductions in total and mean recovered *B. knoxi* and the estimated percentage kill was 96.8% (Table 5.2). The treatment effect was statistically significant (Pearson Chi-square value 31.97, 1 df P<0.001). Of the 20 treated abalone live *B. knoxi* were recovered from 2 shells. One of these has 10 *B. knoxi* chimneys from which 3 live worms were recovered. Most of the shells from each group had small shell blisters (< 5% shell coverage) in the apex region. There was no abalone mortality.

Trial 3. Treatment of B. knoxi-infested stock at Variable Temperatures

Comparison of dead and live worms by Chi-square analysis indicated a significant treatment effect (Pearson chi-square value 48.26, 4 df, P<0.001). The most effective treatment at 21°C reduced the mean *B. knoxi* count to less than 10% of the control count (Table 5.3). Estimates of percentage worm kills were approximately 70% or greater for all treatment except that at 24°C. The shells of stock exposed at 24 °C (71% humidity) did not appear dry at the conclusion of the exposure time and there was no treatment effect (Table 5.3). There was no abalone mortality during treatment or in the week post air exposure. Shell blistering was minimal and was generally less than 5% when present.

Table 5.1 Treatment of severe *B. knoxi* infestation: 3-8 h, 24 °C

	Air Exposure Time (Hours)			
	Control	3	5	8
B. knoxi chimneys ($\overline{X} \pm$ SE)	53.8 ± 7.8	28.1 ± 3.2	45.8 ± 7.7	38.8 ± 7.8
Surviving *B. knoxi* ($\overline{X} \pm$ SE)	49.4 ± 4.6	11.1 ± 1.7	15.4 ± 4.0	3.9 ± 1.4
EI% Kill ($\overline{X} \pm$ SE)	8.9^{A} ± 5.1	62.6^{B} ± 4.1	68.4^{B} ± 4.1	90.2^{C} ± 2.6
Abalone mortality	0/1	0/8	1/7	3/9

n=10 infested shells all treatments except control where n=5
EI%Kill means with shared superscripts are not significantly different (P>0.05)

Table 5.2 Treatment of *B. knoxi* infestation: 4 h, 24 °C

	Total Chimneys	Total Recovered *B. knoxi*	Mean ± SE Recovered *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Abalone Mortality
Control	31	25	1.25 ± 0.19	16.0 ± 6.2	0/20
Treated	35	4	0.20 ± 0.15	96.8 ± 2.1	0/20

n=20 each group, Pearson Chi-square value 31.97, 1 df P<0.001

Table 5.3 Treatment of *B. knoxi* infestation at variable temperatures

Temperature (°C)	Total Chimneys	Total Recovered *B. knoxi*	Mean ± SE Recovered *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Abalone Mortality
Control	32	26	1.24 ± 0.18	19.0 ± 7.9	0/21
15	30	11	0.52 ± 0.17	72.2 ± 9.2	0/21
18	31	10	0.48 ± 0.14	69.4 ± 9.3	0/21
21	35	3	0.14 ± 0.08	91.3 ± 5.2	0/21
24	35	25	1.19 ± 0.20	29.0 ± 8.5	0/21

n=21 all treatments, Pearson chi-square value 48.26, 4 df, P<0.001

Table 5.4 Treatment of *B. knoxi* infestation at 21°C and variable exposure times

Exposure Time (h)	Total Chimneys	Total Recovered *B. knoxi*	Mean ± SE Recovered *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Abalone mortality
Control	17	12	0.75 ± 0.14	33.3 ± 11.8	0/16
1	20	14	0.88 ± 0.18	29.2 ± 11.0	0/16
2	21	8	0.50 ± 0.25	68.8 ± 11.6	0/16
3	23	4	0.25 ± 0.11	81.3 ± 8.7	0/16
4	21	1	0.06 ± 0.06	93.8 ± 6.1	0/16

n=16 all treatments, Pearson Chi-square value 30.27, 4 df P<0.001

Trial 4. Treatment of B. knoxi infested abalone at variable exposure times

Chi-square analysis showed a significant treatment effect (Pearson Chi-square value 30.27, 4 df P<0.001). Trends for recovered worm and estimated kill data showed a potential treatment effect at 2 h and increasing with exposure time (Table 5.4). Only one *B. knoxi* in total survived the most effective 4 h exposure. The relatively high value (33.3%) of the control EI%K indicates derelict *B. knoxi* burrows were common. Mud worm blisters were small when present, covering less than 5% of shell area. There was no abalone mortality during desiccation or in the 3 d post air exposure.

Trial 5. Air drying of old heavily polychaete infested greenlip abalone

Despite the exposure time, temperature and humidity conditions the shells of stock did not appear dry at the conclusion of treatment. There was no statistically significant reduction in numbers of *B. knoxi* (Pearson Chi-square value 1.27, df 1, P=0.260) and clearly no reduction in total polychaetes (Table 5.5). Control EI%K data indicated derelict *B. knoxi* burrows were common. There was one abalone mortality in the treated group during the recovery period. Polychaete worms other than mud worms were very common, with 400 recorded in one treated shell. The majority of the shells were seriously blistered in the range 10-50% blister coverage, frequently with large walled off chambers.

Trial 6. Air drying of recently infested stock under field conditions

Drying resulted in a significant mean reduction of *B. knoxi* from 3.3 to 0.4 per shell under field conditions (Pearson Chi-square value 64.2, df 1, P<0.001-Table 5.6). The EI% Kill value was very high at 87.5%. Small numbers of *P. hoplura* mud worms were present in the control but not in the treatment group. This treatment effect was also statistically significant (Mann-Whitney U Test, U = 120.0, P <0.01). There was no abalone mortality. Mean blister coverage was 4.3% (SD = 7.2%, n = 20)

Table 5.5 Efficacy of air drying for old heavily polychaete infested greenlip abalone

	Control	Treated
B. knoxi chimneys ($\overline{X} \pm$ SE)	10.5 ± 1.7	12.4 ± 2.4
surviving *B. knoxi* ($\overline{X} \pm$ SE)	6.2 ± 1.3	6.4 ± 1.5
EI% *B. knoxi* Kill ($\overline{X} \pm$ SE)	38.1 ± 9.0	52.2 ± 7.8
total worms ($\overline{X} \pm$ SE)	19.5 ± 5.6	71.6 ± 38.0
Abalone Mortality	0/10	1/10

n= 10 each group, Pearson Chi-square value 1.27, df 1, P=0.260 for *B. knoxi.*

Table 5.6 Field trial treatment of recent (< 3 month) mud worm infestation

	Total Chimneys	Total *B. knoxi*	Mean ± SE *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Mean ± SE *P. hoplura*
Control	77	65	3.3 ± 0.5	18.0 ± 6.0	1.0^{A} ± 0.4
Treated	53	7	0.4 ± 0.2	87.5 ± 21.6	0.0^{B} ± 0.0

n=20 for both groups, Pearson Chi-square value 64.2, df 1, P<0.001 for *B. knoxi*
Column means with shared superscripts are not significantly different (P>0.05)

Table 5.7 Field trial treatment of heavily fouled stock with 13 month exposure to mud worm settlement

	Mean ± SE *B. knoxi*	Mean ± SE Small *P .hoplura*	Mean ± SE large *P. hoplura*	Mean ± SE non-spionid polychaetes
Control	1.1^{A} ± 0.2	1.4^{A} ± 0.2	3.0^{A} ± 0.4	12.0^{A} ± 0.8
Treated	0.5^{A} ± 0.02	0.3^{B} ± 0.02	1.2^{A} ± 0.09	2.0^{B} ± 0.13

n=20 treatment group. n=9 control group .
Column means with shared superscripts are not significantly different (P>0.05)

Table 5.8 Short air exposure treatment of three month *B. knoxi* infested stock.

Treatment	Statistic	*B. knoxi* Chimneys	Small *B. knoxi*	large *B. knoxi*
Control	Sum	22	18	5
	Mean ± SE	2.00 ± 1.61	1.64 ± 1.21	0.45 ± 0.69
air dried	Sum	18	1	2
	Mean ± SE	1.38 ± 0.65	0.08 ± 0.28	0.15 ± 0.38

n=11 and 13 for control and treated groups ,respectively
Contingency table analysis: control group 23:0 (live worms: dead worms) and treatment group 3:15 (live worms: dead worms) Pearson Chi-square value 30.22 df 1, P<0.001.

Trial 7. Air drying of heavily fouled, P. hoplura infested stock under field conditions

Due to excess surface fouling the shells of many treated abalone did not appear completely dry at the conclusion of air exposure. Analysis of counts data by Mann-Whitney U Test showed that the reductions in *B. knoxi* and large *P. hoplura* between control and treatment groups (Table 5.7) were not statistically significant ($P>0.05$). However, the reductions in small *P. hoplura* and non-spionid polychaetes were statistically significant ($U = 53.0$, $P=0.03$ and $U =14.5$, $P<0.01$,respectively). Non-spionid polychaetes were common and many appeared to live on the surface of the shell rather than within it.

Abalone mortality data could not be assessed reliably in this trial because after transport to the laboratory for mud worm quantification one control animal and several in the treatment group died or were moribund. It was believed this was due to transport stress and poor water quality in the holding tank. Additionally 10 of 20 abalone in the control group escaped from their holding cage after treatment but before transport. Of the remaining animals, one abalone was excluded from statistical analysis of count data as it contained > 100 *P. hoplura* (mostly post-larvae), a level more than twenty times the mean for the rest of the group. Mean blister coverage for the control group was 20.4% ($SD = 22.6$, $n=10$).

Trial 8. Two and half hour drying of recently B. knoxi infested stock

Although 40 abalone were assigned to each treatment group, subsequent analysis showed that less than half the animals had any *B. knoxi* infestation on the basis of chimney counts. Table 5.8 displays data for the *B. knoxi*-positive abalone only. The 2.5 h air exposure significant reduced infestation (Pearson Chi-square value 30.22 df 1, $P<0.001$). Shell blistering was rare and generally less than 5% coverage when present. There was no mortality in either group (total $n=80$) in the week following treatment.

Trial 9 Drying of 14 month mud worm-infested blacklip abalone

Mud worm counts were reduced in air dried abalone as compared to the control animals for all worm categories. However, none of the reductions (Table 5.9) were statistically significant (P>0.05, Mann–Whitney U Test) except for the category "total small mud worms" (U = 30.0, P = 0.03).

Trial 10. Repeat drying of abalone previously treated one year earlier

Drying resulted in lower counts for all mud worm categories (Table 5.10). These were statistically significant by Mann-Whitney U Test for: total polychaetes (P=0.01), large *P. hoplura* (P=0.01), small *P. hoplura* (P=0.03) and total *P. hoplura* (P=0.01). Reductions were not statistically significant for large and total *B. knoxi*, (P=0.10 and P=0.06), respectively. Insufficient small *B. knoxi* were present for an appropriate statistical test. Mean shell blister coverage was 9.6% (SD=10.0%, n=19). One of the control group animals was lost from the experiment before transport to the laboratory for assessment.

Table 5.11 summarises the results of treatment trials reported above. The column headed "efficacy of treatment" shows treatment as effective only where reductions in worms were statistically significant.

Table 5.9 Treatment of 14 month mud worm infested abalone

Worm count ($\overline{X} \pm$ SE)	Control	Air Dried
Small *B. knoxi*	$0.2^{A} \pm 0.2$	$0.0^{A} \pm 0.0$
Large *B. knoxi*	$2.1^{A} \pm 0.6$	$1.7^{A} \pm 0.7$
Total *B. knoxi*	$2.3^{A} \pm 0.7$	$1.7^{A} \pm 0.7$
Small *P. hoplura*	$0.3^{A} \pm 0.2$	$0.0^{A} \pm 0.0$
Large *P. hoplura*	$7.9^{A} \pm 0.9$	$5.9^{A} \pm 1.2$
Total *P. hoplura*	$8.2^{A} \pm 0.9$	$5.9^{A} \pm 1.2$
Total small mud worms	$0.5^{A} \pm 0.2$	$0.0^{B} \pm 0.0$
Non-spionid mud worms	$5.4^{A} \pm 5.0$	$0.8^{A} \pm 0.5$
Total polychaetes	$16.3^{A} \pm 5.4$	$8.9^{A} \pm 2.0$

n = 10 both groups
Row means with shared superscripts are not significantly different (P>0.05)

Table 5.10 Summer 1999 re-treatment of previously treated stock

Worm count ($\overline{X} \pm$ SE)	Control	Air Dried
Small *B. knoxi*	0.3 ± 0.3	0.0 ± 0.0
Large *B. knoxi*	$3.7^{A} \pm 0.8$	$2.1^{A} \pm 0.9$
Total *B. knoxi*	$4.0^{A} \pm 0.8$	$2.1^{A} \pm 0.9$
Small *P. hoplura*	$0.9^{A} \pm 0.4$	$0.1^{B} \pm 0.1$
Large *P. hoplura*	$3.9^{A} \pm 0.8$	$1.0^{B} \pm 0.5$
Total *P. hoplura*	$4.8^{A} \pm 1.0$	$1.1^{B} \pm 0.6$
Non-spionid mud worms	$1.2^{A} \pm 1.2$	$0.3^{A} \pm 0.2$
Total polychaetes	$10.2^{A} \pm 1.5$	$3.5^{B} \pm 1.4$

n = 9 control group, n=10 treated group
Row means with shared superscripts are not significantly different (P>0.05)

Table 5.11. Summary of trial effectiveness

Trial No.	Infestation (months)	% Shell Blisters	Treatment Time (h)	Efficacy of treatment
1	> 24	>25%	3,5,8	Effective for *B. knoxi*
2	8	<5	4	Effective for *B. knoxi*
3	8-10	< 5	4	Effective for *B. knoxi* 15-21 °C
4	8-10	< 5	1-4	Effective for *B. knoxi* 2-4 hours
5	>36	10-50	4	Ineffective for *B. knoxi* and non-spionids
6	< 3	4.3	3.5	Effective for *B. knoxi* and *P. hoplura*
7	13	20.4	4	Effective: *B. knoxi*, non-spionids, small *P. hoplura* Ineffective: large *P. hoplura*
8	3	< 5	2.5	Effective for small *B. knoxi*
9	14	17.2	3.5	Effective small mud worms only
10	16	9.6	4	Effective: *P. hoplura* all sizes Ineffective: large *B. knoxi*

Effective treatment defined as statistically significant spionid reduction (P<0.05)

5.1.4 Discussion

The body of experimental work demonstrates that air drying of small *B. knoxi* (and small *P. hoplura*) can be highly effective (Table 5.11). Larger mud worms (> 5 mm and typically 10-25 mm) may be susceptible to drying treatment depending on a host of factors including: infestation time, blister severity, stock size and stock fouling. Increasing infestation period allowed mud worms time to become established in burrows deep within the shell. Where larger chambers occurred within blisters survival appeared especially likely. Non-spionid polychaetes were highly susceptible to drying in most trials where they were present. This may be because they tend to occur on the surface of the shell rather than within it.

Appropriate conditions to ensure effective drying are considered to be temperatures >15°C and humidity less than approximately 63%. These conditions are not uncommon on sunny days in Tasmania outside the period late autumn to early spring. Where shells did not fully dry (Trials 5, 7 and one treatment in Trial 3) treatment was ineffective. Lack of drying was associated with high humidity, larger stock and severe shell fouling. The effect of humidity was clearly demonstrated by comparison of results for *B. knoxi* survival dried at 24°C in trials 2 and 3. The treatment was highly effective in trial 2 at low humidity and ineffective in trial 3 at a higher humidity.

Abalone mortality was minimal in the air drying trials, occurring only in trials 1 and 5, which used severely infested stocks. Mortality could not be accurately assessed in trial 7 due to the loss of some animals. It was believed this was due to transport stress and poor water quality in the holding tank. Further work on air-drying and abalone mortality is reported in Chapter 5.2 to follow.

Previous work on treating mud worms in shellfish has focused on oysters. Environmental treatments have included: fresh water soaking (Bailey-Brock and Ringwood 1982, Nel et al., 1996 and Tonkin 1997), heated seawater (Bailey-Brock and Ringwood 1982, Nel et al., 1996) and soaking in hyper-saline water (Mackenzie and Shearer 1959, Bailey-Brock and Ringwood 1982, Tonkin, 1997).

The latter method generally requires heating to dissolve sufficient salts. Leighton (1998) successfully used elevated temperature to treat sabellid polychaete infestation in two relatively warm water abalone species but this approach was unsuitable for the more commercially valuable temperate species *H. rufescens*. There is a paucity of studies where the efficacy of air drying as a treatment has been quantified. However, Whitelegge (1890), Smith (1984) and Nell and Smith (1988) state that removal of Sydney rock oysters from water for ≥ 7 days has been used to treat mud worm infestation in this oyster species. Fortuitously, desiccation is effective as a treatment for infested abalone at much lower exposure times. In relation to this, differences in blister morphology between spionid infested abalone and bivalve molluscs in Tasmania are of interest. In bivalves *B. knoxi* inhabits very large, water filled blisters, whereas, in abalone blisters rarely have a significant volume. These differences are quantified in Chapter 7 (shell blistering section). Thus, blisters typical of *B. knoxi* and, to a lesser extent, *P. hoplura* in abalone may provide relatively poor protection against desiccation strategies.

The use of air exposure as a mud worm treatment is considered a very favourable outcome. The treatment is environmentally benign, uncontroversial compared to potential chemotherapeutic treatments, and requires no withholding period. Compared to potential chemical treatments there is no cost for consumables and handling and labour costs are minimised. The next part of this chapter (section 5.2) examines the effects of air drying on stock health in more detail.

5.1.5 Conclusion

Repeated treatment trails have shown that air drying of abalone is highly effective in reducing levels of mud worms when infestations are relatively recent. Thus, a potential treatment option exists should farmed stock become infested. In susceptible areas the treatment option should be secondary to and compliment avoidance strategies (Chapter 3). Treatment, if required, should be exercised earlier rather than later to maximise mud worm kill rates. Summer appears to be the most appropriate time for treatment as spionid settlement is largely completed and weather conditions are most suitable for effective drying.

5.2 Health effects of air drying abalone

5.2.1 Introduction

The previous section established that air drying of abalone is an effective treatment option for managing mud worm infestations. Mortality of abalone some times occurred following desiccation, but this was limited to heavily mud worm-affected stock. This section focuses on the medium to long-term affects of drying treatment on abalone health. Clearly there is little benefit in treating mud worm infested stock if such treatment leads to subsequent severe growth depression. The affect of air drying treatment on selected physiological parameters is also explored as a means of indicating how long stock require to recover from such treatment.

There is considerable experience in abalone industries of transporting stock out of water from hatchery to grow-out and to market. However, it must be emphasised that air exposure as a spionid treatment involves thorough drying of the shell, not normally associated with stock transport. Previous work on abalone survival out of water has been performed by Whang and Chung (1977). These authors focused on developing safe transportation techniques for 5-10 mm *H. discus* Reeve. There is some evidence that handling, including tagging depresses the growth of abalone. Shepherd and Hearn (1983) noted that greenlip abalone less than 1 year old tagged (using epoxy resin) during hot weather subsequently grew 30% less than stock of the same size tagged in other years. Edwards et al. (2000) found that removing abalone from substrate with a blunt knife depressed growth over a 2 month study period. If mere handling and short term air exposure for tagging can depress abalone growth then, clearly, drying under treatment conditions described earlier (section 5.1) may also depress abalone growth.

The physiology of abalone during air exposure has been examined previously by Wells and Baldwin (1995) by quantifying pyruvate reductase enzymes and adenylate energy charge in *H. iris* and *H. australis*. Tjeerdema et al. (1991) examined the effects of 1 h air exposure in *H. rufescens* in relation to

phosphorylated metabolites and foot muscle pH. This exposure period was thought consistent with natural tidal exposure in some parts of the abalone's range. Watanabe et al. (1994) studied the effect of air exposure on glycolytic metabolites in starved and seaweed fed abalone. Abalone size, exposure times and reported outcomes varied considerably between the previously reported studies. Tjeerdema et al. (1991) found the effects of 1 h emersion were reversed within about 3 h of return to water. By contrast, Watanabe et al. (1994) recorded mortality in air exposed animals and found that starved abalone accumulated less D-lactate and had improved survival. Wells and Baldwin (1995) concluded that stress and probably survival time is related to abalone size. The following experiments seek to define safe air drying-treatment conditions for abalone in the culture environment.

5.2.2 Materials and Methods

Drying and mortality

In a preliminary drying mortality trial 140 blacklip abalone 15-20 mm were obtained from farm 1 (Chapter 2). Twenty animals were used in each of seven treatments comprising an untreated control and six exposure times (1-6 h). Abalone were dried using reverse cycle air conditioning at 21°C ± 0.5°C. Post-treatment mortality was recorded for 17 d while animals were held in small plastic aquaria within the recirculating system (Chapter 2).

A second drying mortality experiment used 156 blacklip abalone (41.5 ± 4.1 mm, mean ± SD) obtained from Huon Aquaculture (Chapter 2). Mud worm infestation was minimal in the group and the animals had been tagged 10 months previously. After 5 d to acclimatise, without feeding, abalone were randomly assigned to 12 baskets with 13 abalone per basket. Experimental treatments consisted of 3 exposure times: 5, 8 and 11 h and an untreated control with three replicates for each treatment. Cages were 200 mm by 200 mm and 250 mm high

with 6 mm mesh bases and plastic sides. Cages were haphazardly arranged in the recirculating holding tank in block design.

Abalone were dried in their cages outside on a cool sunny day 5 April 2000. The temperature varied between 15 and 20 °C and humidity ranged between 40 and 73% (and was less than 65% for all except the initial reading). Following air exposure, abalone in cages were returned to the recirculating seawater system and fed to slight excess (~ 2% body weight.d^{-1}) for 54 days. Water temperature was measure daily and ammonia and nitrite were measured 2-3 times per week using appropriate aquarium test kits (Australian Pet Supplies, Smithfield NSW). Growth comparisons between treatments were made by one way ANOVA on the calculation of SGR length and weight (Chapter 2) for individual animals. Another treatment with an exposure time of 15 h was run concurrently but with an abbreviated recovery period. Two replicates of 15 abalone were exposed over night using reverse cycle air conditioning. Temperature range was 17-21 °C and humidity was 65%. Abalone were then returned to water and housed as described above for 2 weeks, during which, mortality but not growth was recorded.

Drying and long term growth

Two hundred blacklip abalone, mean length 39.3 mm (SD = 4.7 mm) and weight 8.9 g (SD = 3.3 g), were obtained from an east coast, land-based farm (referred to hence as farm 2). All the abalone were tagged and 100 (in 2 replicates of 50) animals were assigned to either an untreated control group or a drying treatment group. Air-dried abalone were placed in the shade on a sunny day for 4 h; the temperature range was 16-20.5°C and the humidity ranged between 57-65% except for a short interval at 69%. Replicates were dried on separate sheets of plastic in the same general location and were assigned to separate "hides" in a commercial scale grow-out tank. Control animals were immersed immediately after tagging; each replicate was placed in a separate hide. As many tagged experimental abalone as possible were recaptured and re-measured 168 d following treatment.

A similar experiment was conducted on 6 October 1999 at Tasmanian Tiger Abalone, Dunalley (Chapter 2) using greenlip × blacklip hybrids. A total of 200 abalone, mean length 30.4 mm (SD = 5.6 mm) and mean weight 3.8 g (SD = 1.7 g), were tagged for treatment with replication as above. Animals were exposed for 3 h with a temperature range of 15-17°C and a humidity range of 60-84%. Abalone were placed in direct sunlight for up to 10 min at a time to ensure thorough drying. Treatment and control groups were returned to a commercial scale grow-out tank with 50 treated and 50 untreated animals assigned to each of 2 replicate hides. After a 214 d growth period experimental abalone were recaptured and re-measured.

Experiment 3 commenced at Huon Aquaculture 15 February 2000 using abalone already present on the farm for 6 months and 2 years old. Mean length and weight (± SD, n=146) were 37.4 ± 4.1 mm and 7.3 ± 2.2 g, respectively with 78 animals in the treated group 64 in the control. Air exposure time was 2.5 h with a temperature and humidity range of 20-21 °C and 56-64%, respectively. The on-growing period was 210 d during which abalone were grown in Aquatech© Trays (Chapter 2) with 39 air-dried and 32 control animals in each of 2 trays. The null hypothesis in the long term growth trials was that there would be no difference in growth between air-dried and control stocks. Statistical analysis for the three growth comparison trials consisted of Specific Growth Rate comparisons for weight and length between treatments.

Clinical pathology and histology methods

Serum samples and excised tissue sections (including gills, digestive gland, right kidney and foot) were collected from abalone used in Trials 2 & 9 (section 5.1) immediately after drying, and following a recovery period, to assess the effects of drying. Recovery periods were 1 week and 1 day for Trials 2 and 9, respectively and drying times were 4 and 3.5 h, respectively (refer to section 5.1 for full treatment history).

Whole shucked abalone were preserved in 10% seawater formalin and tissue samples processed with haemotoxylin and eosin staining by standard tissue processing methods (Chapter 2).

Samples of haemolymph were withdrawn from the cephalic sinus using a 27 gauge needle attached to a 1 ml syringe (Chapter 2). Approximately 0.3-0.6 ml of fluid was removed, centrifuged at 1800 *g* for10 min, frozen at -10°C and analysed by Cobas Mira auto-analyser (Animal Health Laboratory, DPIWE, Tas.) for Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, protein and glucose. Copper was analysed using standard atomic absorption methods (Chapter 2). Haemocyte counts were quantified using a haemocytometer and converted to cells per ml (Chapter 2).

The effect of drying treatment on haemolymph pH and weight loss was examined in another experiment. Abalone were obtained from Huon Aquaculture and maintained at the University of Tasmania, Launceston (Chapter 2) at 15-16 °C. Stock were approximately 2 years old with mean length of 42.8 mm (SD =3.7 mm, n= 37) and mean weight of 11.9 g (SD= 2.9, n=37). Abalone were air-dried for 5 h at a temperature and humidity range of 14-16 °C and 41-59%, respectively.

Wet weights of all abalone were recorded as drying began and samples of abalone were re-weighed periodically as drying continued. Animals were also weighed periodically during the recovery time. Samples of haemolymph pH were taken during drying and recovery phases from both the cephalic sinus and the foot as described previously. The pH was measured using an Activon AEP333 probe connected to a Cameron Instrument Company (Port Aransas Texas, USA) BGM 200 blood gas analyser. The null hypotheses were that there would be no difference between haemolymph pH and wet weight of abalone at different sample times during and after the drying treatment.

5.2.3 Results

Mortality trials

Mortality of 15-20 mm stock dried at 21°C was minimal with no deaths for control, 1, 2, 3 and 5 h air exposure treatments (Table 5.12). In the second experiment no mortality was observed in the 8 week period following air exposure for 11 h (Table 5.13). Growth was poor in all treatment groups including the non-dried control (0.89 mm ± 0.76 mm, 1.69 g ± 1.21 g; $\overline{X}$ ± SD, n=146). There was no significant difference between SGR of treatments for length ($F = 1.40$, df 3,140, $P = 0.246$, ANOVA) or for weight ($F = 1.39$, df 3,140, $P = 0.249$, ANOVA).

Tank conditions were not ideal during the recovery period: temperature extremes of 10-20 °C were recorded with a mean daily reading of 15.0°C (SD = 2.1°C) and un-ionised ammonia levels reached 0.25 $mg.l^{-1}$. One dead abalone had a discoloured lesion on the foot believed to have been a knife wound. There were a few escapes from the experimental cages and/or holding tank. Of 30 abalone air-dried for 15 h on 4-5 April 2000, most appeared moribund at completion of drying but subsequently recovered with only 1 mortality in the 2 week recovery period.

Table 5.12. Mortality data: 1- 6 h exposure, 15-20 mm abalone

Cumulative mortality	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
initial	0/20	0/20	0/20	0/20	0/20	0/20
17 days	0/20	0/20	0/20	1/20	0/20	2/20

Table 5. 13. Mortality data: 5-11 h exposure, 42 mm abalone

Cumulative mortality	Control	5 hours	8 hours	11 hours
initial	0/39	0/39	0/39	0/39
54 days	1/39	2/37	1/39	0/39

Table 5.14 Clinical pathology indicators for 4 h air-dried abalone (means ± SD)

	Untreated control	Immediately post Treatment	7 days post treatment
Cu^{2+} ($\mu mol.l^{-1}$)	$244^{A} \pm 56$	$288^{A} \pm 58$	$243^{A} \pm 100$
Na^{+} ($mmol.l^{-1}$)	$446.6^{A} \pm 6.7$	$432.0^{B} \pm 7.7$	$446.4^{A} \pm 7.7$
K^{+} ($mmol.l^{-1}$)	$12.8^{A} \pm 1.0$	$14.7^{B} \pm 0.8$	$12.7^{A} \pm 1.3$
Na^{+}/K^{+} ratio	$35.2^{A} \pm 3.1$	$29.6^{B} \pm 1.4$	$35.5^{A} \pm 3.5$
Cl^{-} ($mmol.l^{-1}$)	$515.3^{A} \pm 13.1$	$509.8^{A} \pm 10.6$	$515.9^{A} \pm 11.0$

n=10, except immediately post treatment sampled group, n=5

* Row means with shared superscripts are not significantly different ($p>0.05$)

Table 5.15 Clinical pathology indicators for 3.5 h air-dried abalone from two bleed sites (means ± SD).

		Neck sinus			Foot	
Units	Control	Post-treatment	24 h post treatment	control	Post-treatment	24 h post treatment
Cu^{2+} ($\mu mol.l^{-1}$)	366 ± 94	514 ± 107	352 ± 32	362 ± 83	508 ± 98	352 ± 39
Cl^{-} ($mmol.l^{-1}$)	496.4± 16.1	529.2 ± 13.8	491.8 ± 5.2	495.8 ± 4.8	521.0 ± 7.7	484.0 ± 10.5
K^{+} ($mmol.l^{-1}$)	10.7 ± 0.4	13.1 ± 0.5	11.0 ± 0.7	12.0 ± 1.0	14.3 ± 0.7	12.4 ± 1.6
Na^{+} ($mmol.l^{-1}$)	458.0± 19.9	501.2 ± 11.1	459.6 ± 2.9	455.0 ± 0.0	490.6 ± 7.3	455.0 ± 6.0
Na^{+}/K^{+} ratio	42.8 ± 0.8	38.4 ± 1.1	42.0 ± 2.4	38.0 ± 3.1	34.6 ± 1.5	37.0 ± 4.8
Ca^{2+} ($mmol.l^{-1}$)	11.2 ± 0.7	12.3 ± 0.3	11.4 ± 0.2	11.1 ± 0.3	12.0 ± 0.3	11.2 ± 0.4
Mg^{2+} ($mmol.l^{-1}$)	51.2 ± 2.4	55.5 ± 1.3	50.7 ± 0.9	49.6 ± 2.0	54.9 ± 1.2	49.8 ± 1.2
Glucose($mmol.l^{-1}$)	0.22 ± 0.04	0.34 ± 0.11	0.26 ± 0.10	0.26 ± 0.11	0.40 ± 0.14	0.28 ± 0.10
Protein ($g.l^{-1}$)	10.3 ± 1.9	13.9 ± 2.8	9.1 ± 1.4	10.0 ± 1.7	14.0 ± 3.1	10.1 ± 0.9
Haemocytes.ml^{-1} ($\times 10^{6}$)	6.8 ± 2.6	10.1 ± 2.4	6.3 ± 1.7	-	-	-

n=5 all data except for 24 h neck sinus bleed Cu data where n=4

Growth of air-dried abalone

In experiment one, 41 tagged blacklip abalone (11 control, 30 air-dried) were recovered from the grow out tank at farm 2. A mortality episode over the summer of 1999/2000 reduced abalone numbers, generally, and affected recovery of experimental abalone. Five deaths occurred in the air-dried group in the week following treatment. There was no significant difference between the groups in SGR for length (t=-0.69, df 39, p=0.497 t -Test) nor for SGR weight (t=-0.43, df 39, p=0.669 t -Test). Growth in length (pooled treatments) was 13.1 ± 3.4 mm and weight increment was 15.0 ± 4.9 g ($\overline{X}$ ± SD, n = 41 both data sets).

In experiment two, at Tasmanian Tiger Abalone 62 of the hybrid experimental abalone (42 control, 20 air-dried) were recovered 214 d after commencement of the trial. As at Marine Shellfish Hatcheries, a mortality episode at the farm significantly reduced stock numbers in some tanks, including the tank housing the trial stock. There was no significant difference in SGR of air-dried and control stock for length (t=-0.77, df 59, p=0.447 t-test) or for SGR weight (t=-0.25, df 60, p=0.804 Unpaired t-test). Growth in length and weight (pooled treatments) were 9.9 ± 2.5 mm (X ± SD) and 5.3 ± 2.6 g (X ± SD), respectively.

In experiment three, at Huon Aquaculture 78 abalone (42 control, 35 dried) were recovered 210 d after drying. Treatment group animals appeared stressed immediately after air exposure, with limited clinging and turn-over ability. However, there were no dead animals in the weeks immediately following drying treatment. After 210 d there were 5 dead abalone in the control treatment and 3 in the air-dried group. Mean length and weight of control animals increased 4.0 mm (SD= 2.0, n=42) and 3.4 g (SD=1.5, n=42) , respectively. Mean length and weight increment for dried abalone were 3.2 mm (SD=1.5, n=35) and 2.6 g (SD=1.8, n=35): a reduction of approximately 20% for length and 24 % for weight, respectively as compared to the control group. Comparison of SGR by unpaired t–test found significant differences for length (t=2.02, df 76, p=0.047) and weight (t=4.55, df = 75, p<0.001).

Clinical Pathology

Haemolymph concentration of electrolytes and copper changed immediately following 4 h air exposure (Treatment Trial 2, section 5.1) and recovered to pre-treatment levels when sampled 7 d later (Table 5.14). Analysis by ANOVA indicated a significant difference for Na^+ (F ratio 6.89, df 2 22 P=0.005), K^+ (F ratio 5.46 df 2 22 P=0.012) and Na^+/K^+ ratio (F ratio 6.37 df 2 22 P=0.007). Samples taken immediately post-treatment were significantly different from the control and samples taken 1 week post treatment. Changes evident immediately after treatment were not statistically significant for Cu^{2+} and Cl^- by ANOVA (F ratio 0.57 df 2 22 P=0.576; F ratio 0.43 df 2 22 P=0.654 for Cu^{2+} and Cl^-, respectively).

In abalone dried for 3.5 h (Treatment Trial 9, section 5.1) haemolymph concentrations of blood ions, protein and glucose from both the cephalic sinus and the foot rose following air exposure and recovered to pre-treatment levels when sampled 24 h later (Table 5.15). Haemocyte counts taken from the cephalic sinus followed the same trend. Data were analysed using a two-way ANOVA with sample time and sample site as fixed terms (Appendix 5A). Sample time was statistically significant for all variables (except haemocyte count, P=0.052) and site was significant (P<0.05) for Na^+ and K^+ (Appendix 5A). The mean control level for circulating haemocyte counts withdrawn from the cephalic sinus was 6.8×10^6 cells.ml^{-1} (Table 5.15). "Normal" haemocyte counts for uninfested abalone using the foot cut method (Chapter 2) are given in Appendix 5B.

Histological changes as a result of air drying treatment were few. Abalone in both drying trials showed increased gill mucus between filaments in samples fixed immediately post exposure. Increased numbers of vacuoles were seen in a small number of individual filaments following desiccation but were not considered elevated overall. Changes in other organs following treatment were not observed.

In the drying trial where weight loss of abalone was measured, mean percentage weight loss increased with time out of water to a peak at 8.1% after 5 h, dropping to 1.5% when sampled nearly 2 d later (Figure 5.1). One-way ANOVA of weight loss variation was statistically significant (F ratio 14.94 df 6 104 $p<0.001$). Two data points with high residuals were deleted prior to ANOVA. Concurrent sampling of haemolymph pH from two sites showed a drop in pH while animals were out of water, followed by an increase to greater than original values upon return to water (Figure 5.2). Two-way ANOVA was significant for time as a factor ($P<0.001$) but not bleed site ($P>0.05$). The ANOVA table is shown in Appendix 5C.

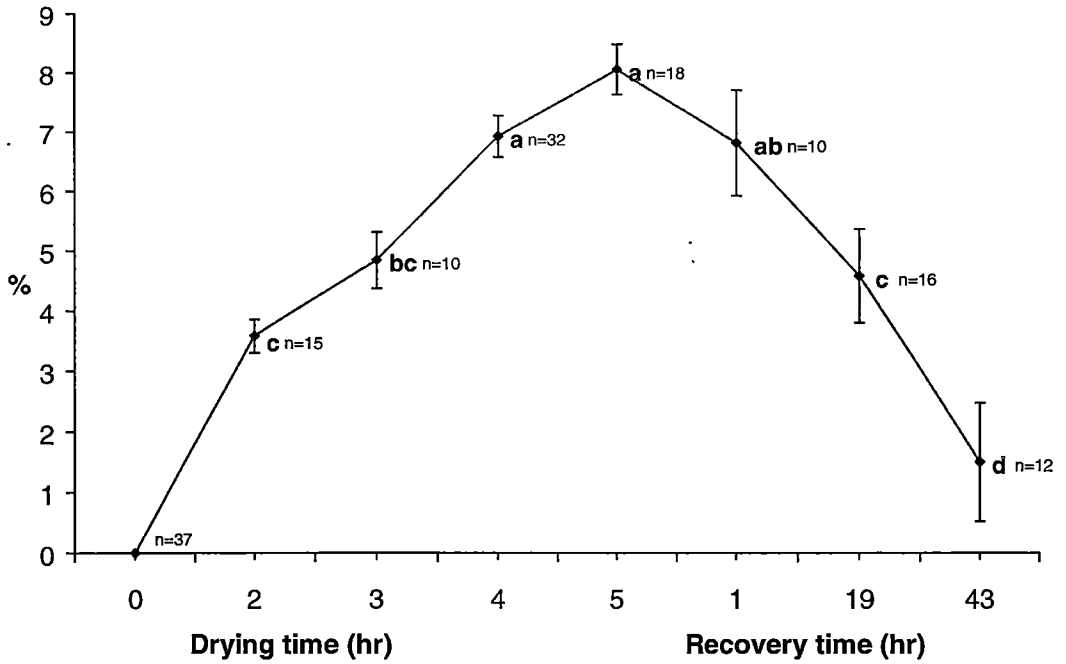


Figure 5.1 Percentage weight loss for 5 h air dried and recovering abalone. (Means ± SE, n values displayed. Means with shared superscripts not significantly different, P>0.05)

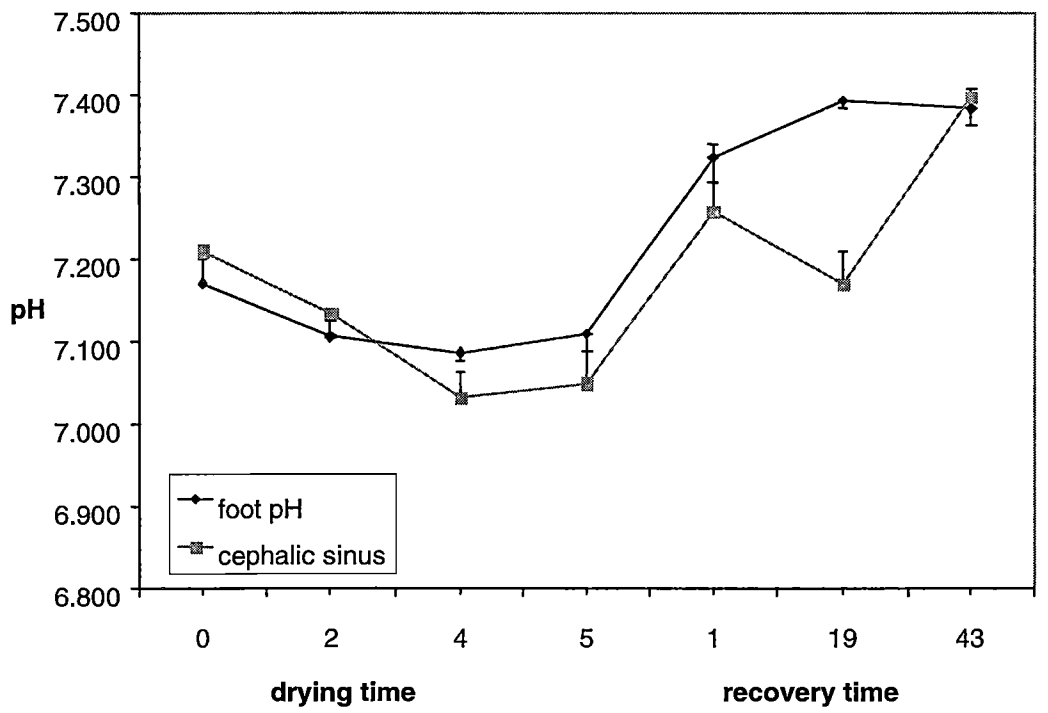


Figure 5.2 Haemolymph pH sampled from two sites during 5 h air drying and recovery (Means ± SD, n =5, except initial time n=10)

5.2.4 Discussion

Treatment trials performed in section 5.1 showed that mud worm infestation rates for lightly infested abalone were considerably reduced by air drying for 2-4 h. Post-drying recovery times of approximately a week failed to result in any abalone mortality in lightly infested stocks. Longer air exposure and recovery times were used in follow-up experiments described in this section (5.2) with the intention of defining a safe period for treatment air exposure. Abalone proved surprisingly tolerant of air drying. There was no mortality of 15-20 mm stock dried for up to 3 h and of 40 mm animals dried for 11 h, providing ample scope for effective spionid treatment. This contrasts with poor survival for smaller abalone (5-10 mm) air-dried in the shade at 24°C (Whang and Chung 1977). Wells and Baldwin (1995) found that larger abalone of two New Zealand species tested were "less susceptible to anaerobic stress than smaller animals". The larger abalone conserved relatively more adenylate energy charge and had lower concentrations of the glycolytic end products lactate and tauropine. It was considered beyond the scope of the present research to establish the point at which air drying abalone results in significant deaths of animals.

There was no difference in growth of control and air-dried (5, 8, 11 h) abalone 54 d post treatment. However, growth was poor in the control group and temperature fluctuation was a problem. Ammonia levels were potentially high enough to suppress growth according to data of Harris et al. (1998). Thus, the effect of air drying on growth may have been masked by other factors. Edwards et al. (2000) has shown that removal of abalone from the substrate with a knife is enough to depress the growth rate over a 2 month period.

The series of three experiments on medium-long term post drying growth effects produced conflicting results. Two trials showed no growth depression but the third trial demonstrated growth depression of 20-25%. It may be significant that stock in the trial showing reduced performance were growing slowly before the trial began and untreated control animals continued to perform relatively poorly.

The recorded growth depression was similar to that reported by Shepherd and Hearn (1983) for wild juvenile abalone handled in hot weather.

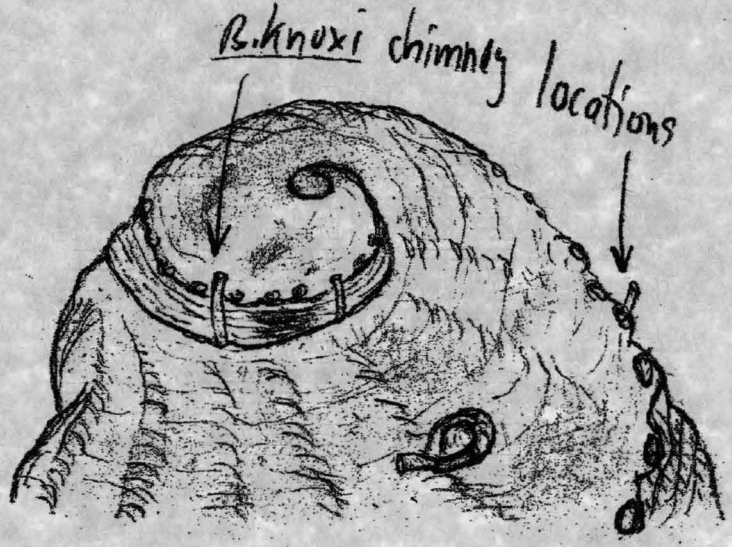
Given the potential for an air drying treatment to suppress growth of stock this management option should be used prudently with the emphasis on avoidance of infestation. Where treatment is required, stress to abalone will likely be minimised by early intervention, reduced drying time, reduced air temperature and reduced differential between sea and air temperatures. Additionally, Watanabe et al. (1994) found that starved abalone survived air exposure at considerably greater levels than fed animals.

Measurement of haemolymph electrolytes and pH have provided baseline data on normal levels for abalone (explored further in Chapter 8 in relation to spionid infestation and osmoregulation). Serum analysis showed the various measured parameters return to the levels designated as "normal" within 24 h of return to water. Levels of measured variables generally rose as a result of desiccation. This may simply indicate loss of water from tissues (measured at 8% after 5 h in the weight loss experiment). However, the Na^+/K^+ ratio fell significantly in both experiments indicating a differential rate of change between these electrolytes. A return to normal physiological conditions within 24 h is consistent with Gade (1988) who showed that the adenylate energy charge in shell adductor and foot muscle recovered to normal levels within approximately 4 h of return to water. Gade (1988) studied 5-7 cm *H. lamellosa* and anoxia was produced using de-oxygenated water rather than air exposure. Tjeerdema et al. (1991) found that in 9 cm *H. rufescens* the effects of 1h air exposure were reversed within 3 h with respect to intracellular foot pH and phosphoarginine. The trend seen in the present study for pH to rise to values above the starting point after return to water was seen in 1 of 3 abalone studied by Tjeerdema et al. (1991).

The weight loss trial indicated that abalone undergo dehydration when removed from water. If weight loss was solely due to drainage of gill cavity water then it would be expected that initial weight would be recovered rapidly on re-immersion. This was not the case. Two hour air exposure resulted in approximately half the weight loss of the 5 h exposure period. Thus, shorter exposure periods, still adequate to reduce spionid infestation (section 5.1) are recommended to minimise stress to stock.

5.2.4 Conclusion

Research presented in Chapter 5 (sections 1 & 2) has shown abalone can survive air exposure for times well in excess of those required to significantly reduce mud worm infestation. Two to four hour air-drying treatment at humidity less than about 63% significantly reduces spionid infestation, without medium to long term impact on abalone mortality. There was, however, some evidence that such treatment can potentially reduce stock growth by 20-25% in the long term. Air drying treatment should, therefore, be used judiciously. It is recommended that mud worm avoidance strategies should be practised in the first instance. Treatment may be more suited to situations where mud worm settlement is relatively severe. Where practised, early intervention allows reduction of exposure times, minimising abalone stress.


B. knoxi chimney locations

Frontispiece: *Boccardia knoxi* chimneys on abalone shell

Chapter 6
RISK FACTORS

General Introduction

In Chapter 3 the timing of mud worm reproduction and larval settlement was described allowing the risk of spionid infestation to be minimised by avoidance strategies. In this chapter physical characteristics of abalone shells including size, presence of fouling organisms and morphological differences between species are examined in relation to degree of spionid infestation. Characteristics of the culture situation with respect to aspects of rearing vessel design and location in the water column are also assessed with respect to risk of mud worm infestation. The chapter general discussion includes a review of environmental characteristics that are important indicators of spionid impacts.

6.1 The effect of abalone size on mud worm settlement

6.1.1 Introduction

During the first season of mud worm settlement temporal studies, it was noted that stock placed in August 1998 became considerably more infested than stock placed 1-2 months later within the same settlement season. It was suspected stock size and shell fouling were important factors in the differential spionid infestation. The August 1998 transferred stock were larger with more shell fouling than subsequent intakes. The effect of abalone size in mud worm recruitment is examined in this section and that of shell fouling in section 6.2.

The role of mollusc size with regard to shell boring has been examined previously by Hansen (1970), Clavier (1989) and Caceres-Martinez et al., (1999). Hansen (1970), in a study of boring sponge (*Clione celata* Grant) and boring clam (*Penitella conradi* Valenciennes) infestation of wild abalone found that such infestations increased with increasing host size.

The findings of Clavier (1989) were similar in relation to boring sponge, *Polydora* sp. and an abalone host species. Caceres-Martinez (1999) found a trend for increasing *Polydora* sp. infestation with increasing size of the clam host. A study of wild *H. diversicolor* by Kojima and Imajima (1982) also found increasing *Polydora* sp. infestation with increasing host size. Knowledge of any differential spionid infestation risk based on size in the culture situation is considered useful in managing transfer of abalone from hatcheries to potentially mud worm susceptible sea based grow out sites.

6.1.2 Methods and Materials

Experimental animals

Black lip abalone of three different size cohorts were selected from available stock at farm 1 (Chapter 2). Stock cohorts were approximately 6 months, 18 months and 3 years old and measured 15.0 ± 0.4 mm (n=23), 34.3 ± 0.5 mm (n=100) and 50.9 ± 0.7 mm (n=20), ($\overline{X}$ ± SE), respectively. These size groups hence forth referred to as "small", "medium" and 'large" were considered to represent the extremes and middle of the range at which sea based grow out farms are likely to purchase hatchery stock.

Experimental protocol

Fifty stock of each size class were placed in 4 "basket" type culture cages (Chapter 2). Two cages were hung within 2 m of each other at both Aquatas and Huon Aquaculture study sites. The experiment began July 21-22 1999 and was concluded 16 February 2000 at Aquatas and 15 March 2000 at Huon Aquaculture. This was intended to expose the stock to one *B. knoxi* settlement period (later shown to be September 1999 to November 1999 – Chapter 3). Some abalone were removed before the completion of the experiment to provide data on timing of mud worm settlement.

At completion abalone were transported to the laboratory in seawater. Preliminary investigation showed low rates of mud worm settlement, therefore, abalone were shucked and shells exposed to standard vermifuge solutions (Chapter 2) in replicates of five. Counts of *B. knoxi* chimneys and expelled *B. knoxi* and *P. hoplura* worms were made. Samples from shells of each size class were assessed individually for mud worm shell damage by the percentage blister and Subjective Shell Damage Rating (SSDR) methods (Chapter 2). The surface area of a sample of shells from each size class was estimated by the blister tracing method. A subjective shell fouling rating (0-3) was also given for random samples of shells in each size class. Shells without fouling organisms as determined by gross examination were given a zero rating and heavily fouled shells given a rating of "3" (Figure 2.4, Chapter 2 shows rating "3" shells). The null hypothesis was that size of abalone would have no effect on settlement by spionids, any subsequent shell damage or settlement by other fouling organisms.

Statistical Analysis

Basket replicates for each study site were assessed for each size class and measured variable (*B. knoxi* chimneys, *B. knoxi* worm counts, *P. hoplura* worm counts, SSDR, % blister coverage and subjective fouling score) by Mann-Whitney *U* Test. Where replicate variables were not significantly different ($P>0.05$) they were combined for further assessment. Comparisons between size classes were made by Kruskal-Wallis test for each of: *B. knoxi* chimneys, *B. knoxi* worm counts, *P. hoplura* worm counts, SSDR, % blister coverage and subjective fouling score. Mean separations for significant Kruskal-Wallis tests were performed by the method of Zar (1984).

6.1.3 Results

At the Aquatas site mud worm settlement as measured by the presence of *B. knoxi* chimneys, *B. knoxi* worms and *P. hoplura* worms increased generally with increasing stock size class (Figure 6.1). The large size class was significantly different ($P<0.05$) from the other size classes (individual Kruskall-Wallis statistics given in Appendix 6A).

Similarly, at Huon Aquaculture the largest stock size acquired significantly more mud worm settlement using all measures than medium and small stock size categories ($P<0.05$, Appendix 6B). Figure 6.2 displays this data with replicates separate as *P. hoplura* data could not be grouped in the medium size class ($P<0.01$ $U = 0.0$, Mann-Whitney U Test) and because the small size class category was deleted from replicate 2 due to poor recovery of animals. Settlement of *B. knoxi* on experimental stock at both sites was considered low with a maximum of approximately 8-12 chimneys per replicate of 5 large size class abalone. Survival of *B. knoxi* worms until assessment in February – March was even lower at about half the levels indicated by chimney count data (Figures 6.1 and 6.2). In the two smaller stock size classes *B. knoxi* settlement levels were at least 5 times lower than those in the large size class. The majority of individual abalone in these size classes experienced no *B. knoxi* settlement. The surface areas of abalone from each size class were: 292 ± 44, 1098 ± 153 and 2240 ± 85 mm^2 ($\overline{X} \pm$ SD, n=5) for small to large animals, respectively.

Shell damage attributable to mud worms was significantly higher ($P<0.05$, Kruskall-Wallis test) in the larger stock size class at both sites as measured by both percentage blister cover and the SSDR methods (Figures 6.3 and 6.4), (individual statistical comparisons given in Appendix 6C). Blister damage was 1% or less in the small and medium stocks. In the largest stocks, mean shell blister cover values were 9.0% (SE=1.7) and 13.3% (SE=1.3) at Aquatas and Huon Aquaculture, respectively.

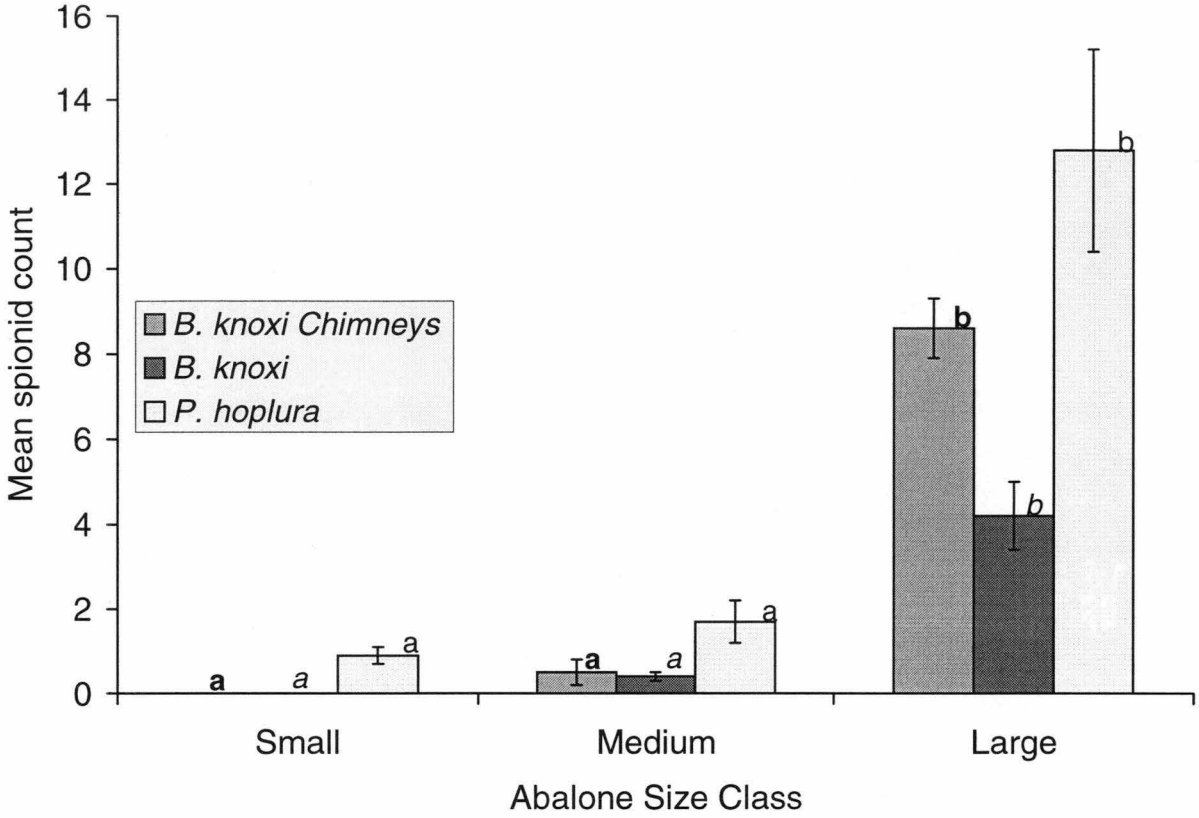


Figure 6.1 Mud worm settlement on 3 size classes of abalone at Aquatas. Means are totals per replicates of five abalone ± SE, (n = 14, 17 and 20 for small, medium and large classes, respectively). Data sets with shared indicators are not significantly different from one another (P>0.05).

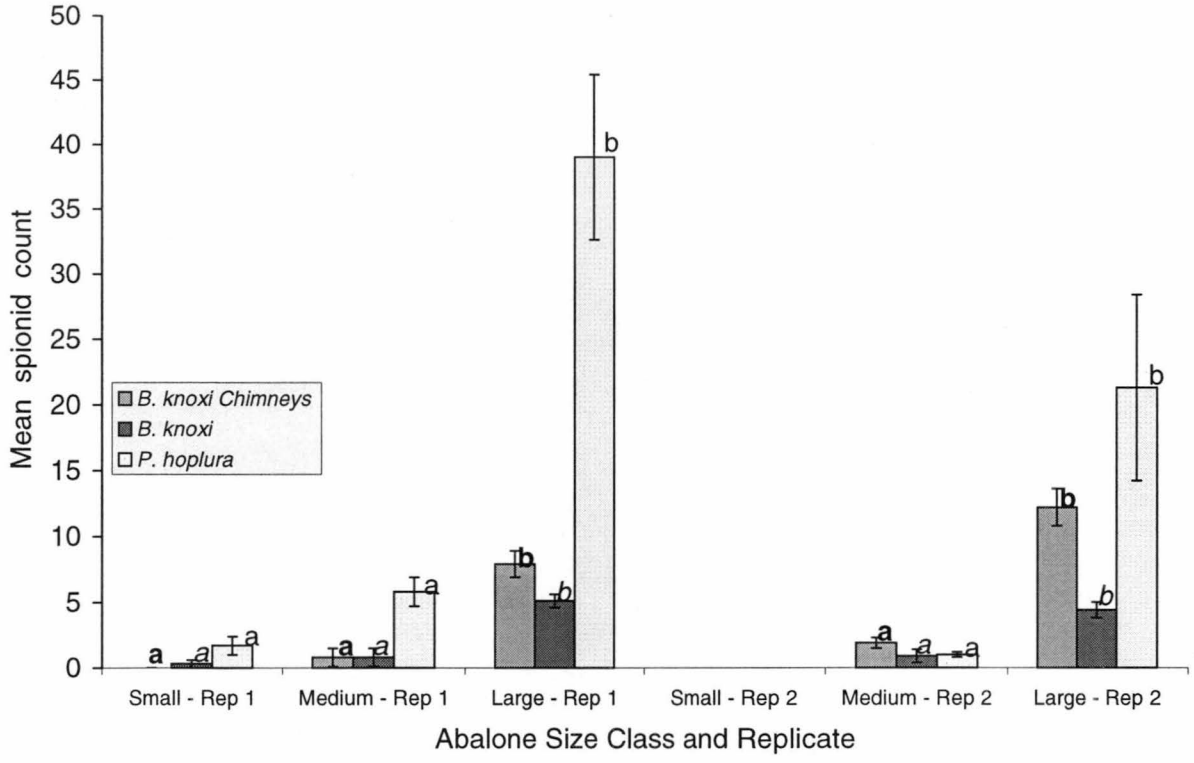
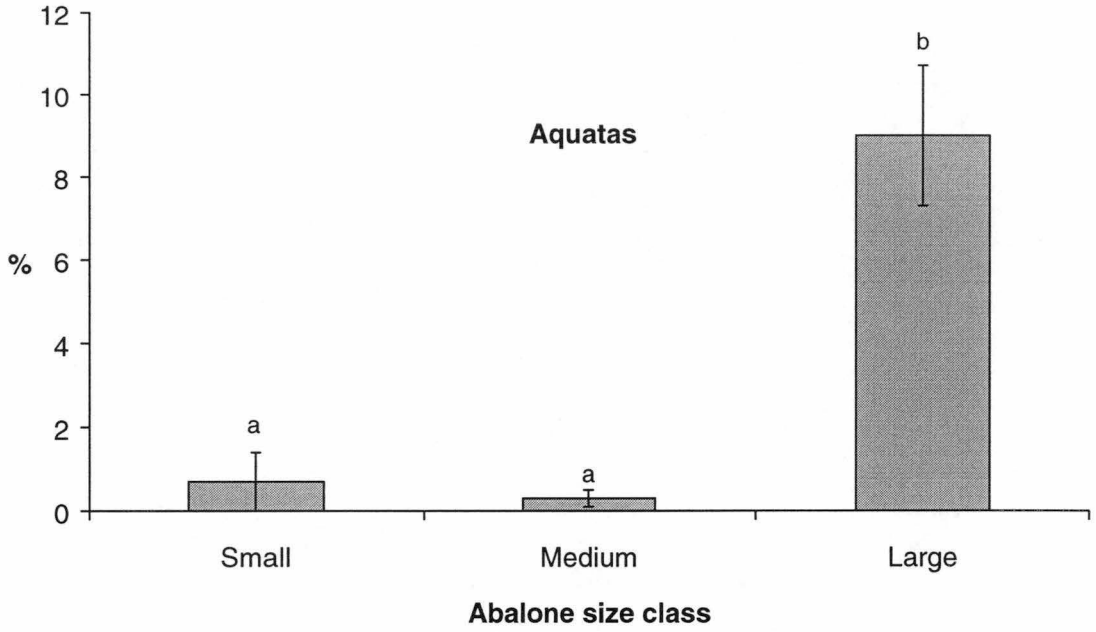


Figure 6.2 Mud worm settlement on three size classes of abalone at Huon Aquaculture. Means are replicates of five abalone ± SE, (n = 3, 6 and 9 - replicate 1 and n = 0, 7 and 10 - replicate 2; small, medium and large classes, respectively). Data sets with shared indicators are not significantly different from one another (P>0.05).



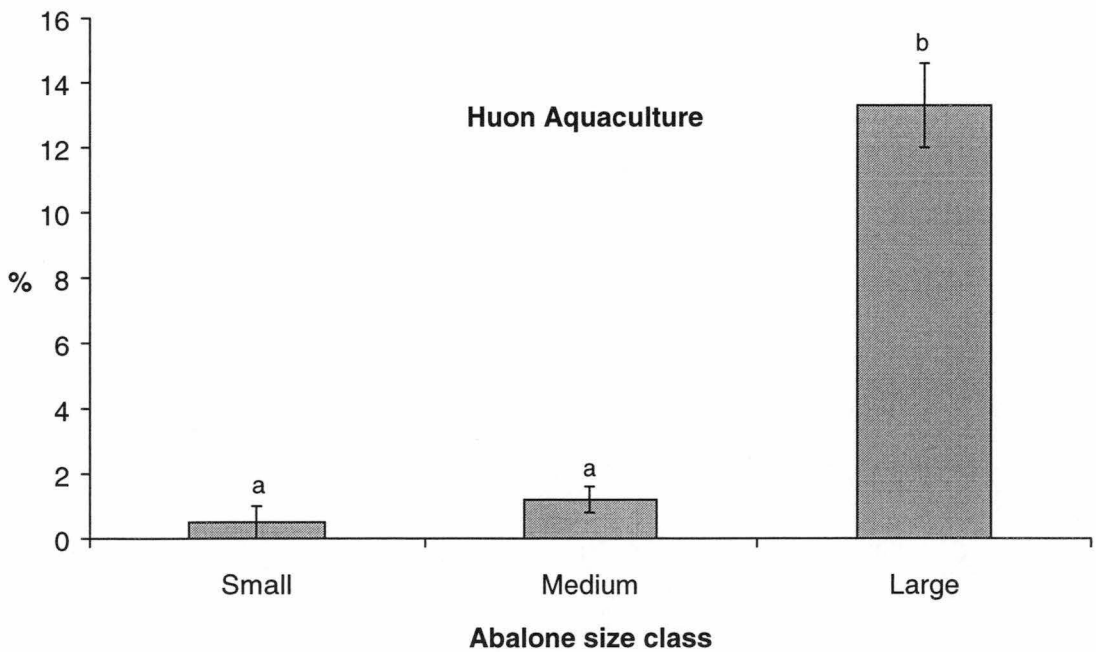
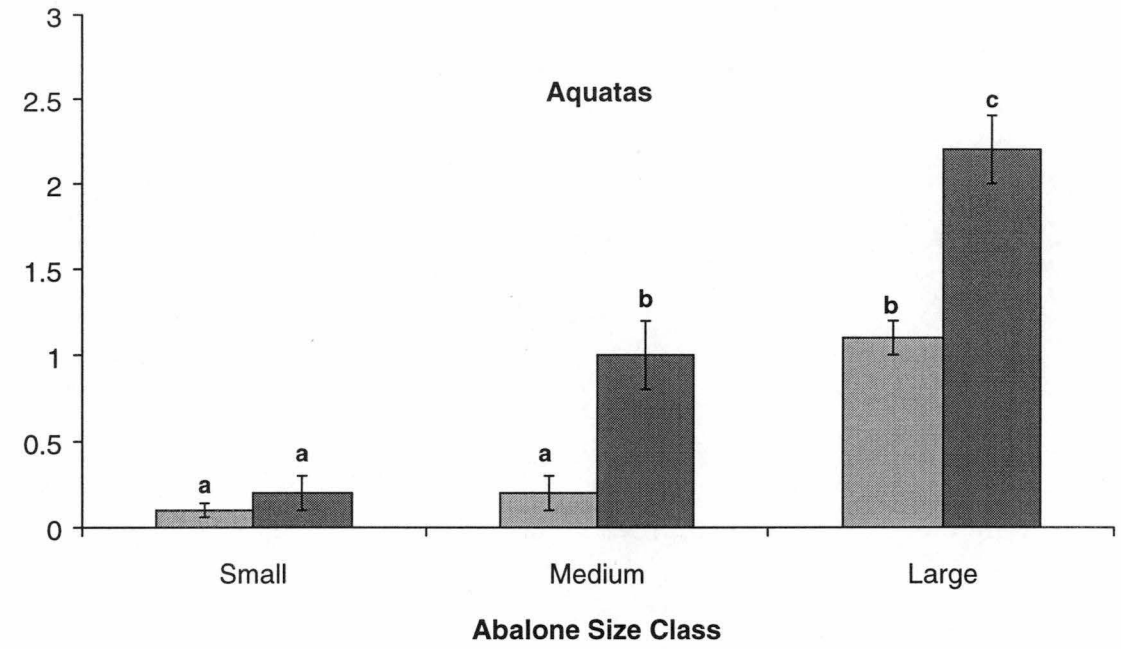


Figure 6.3 Percentage of shell area infected by mud worm blisters. Mean ± SE, (n = 20 all Aquatas data; n = 25 Huon Aquaculture, except n = 10 small size class). Data sets with shared indicators not significantly different (P>0.05).



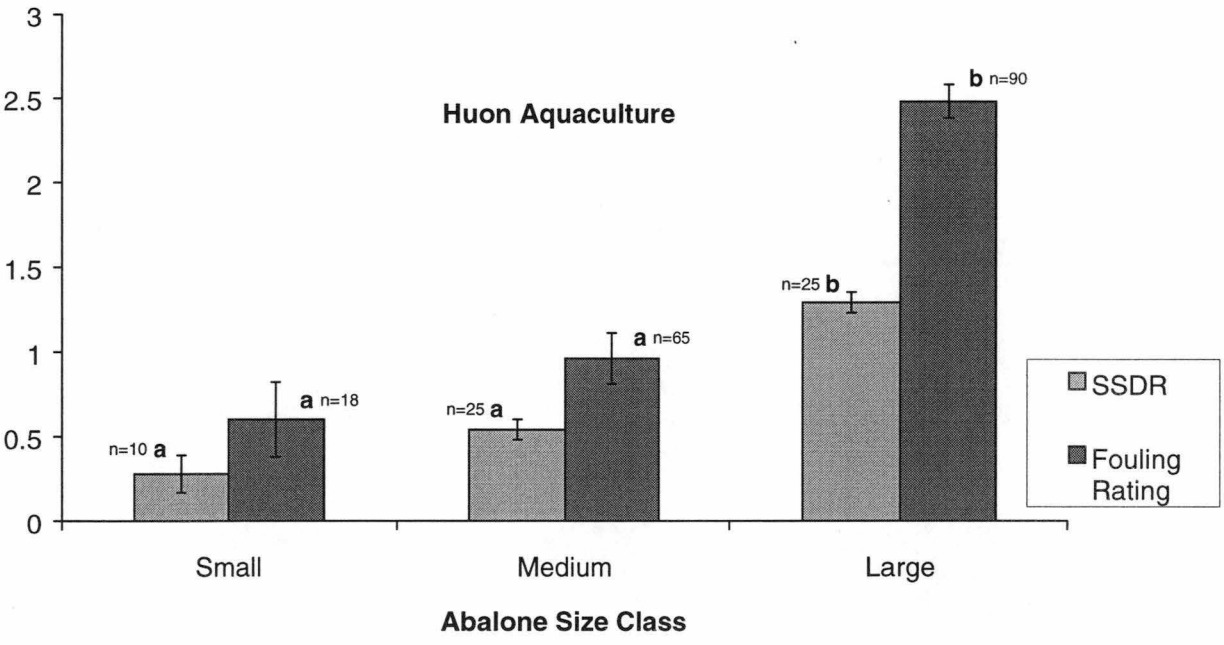


Figure 6.4 SSDR and fouling data. . Mean ± SE, (n = 20 all Aquatas data, n shown for Huon Aquaculture data). Data sets with shared indicators not significantly different (P>0.05).

Subjective fouling data which, in this context, were a measure of settlement for spirorbids, *Pomatoceros* sp. (Figure 2.4, Chapter 2), and Pacific oysters showed the same trend as mud worm settlement data – increasing with stock size. At Aquatas, each size class was significantly different from one another ($P<0.05$, Kruskall-Wallis test, Appendix 6C). At Huon Aquaculture, fouling values for the small and medium size classes were not significantly ($P>0.05$) different from each other but both values were significantly less ($P<0.05$) than fouling ratings for larger abalone (Figure 6.4), (Appendix 6C).

6.1.4 Discussion

Settlement rates of *B. knoxi* were low in this experiment with means of less than one worm per replicate of five abalone in small and medium size classes at both farms. The shell surface area approximately doubled between the medium and large size class abalone, and spionid infestation increased by a factor of about five. This suggests mud worm settlement may not be simply passive and could be influenced by spionid behaviour eg. settlement cues.

The significance of stock size on mud worm settlement has rarely been mentioned in cultured mollusc mud worm studies. One exception is Handley (1997) who suggested that small oyster stock were not attractive for settlement as a possible explanation for their failure to become infested with *B. knoxi*. Studies of wild abalone and spionids have also shown increasing infestation with increasing size (Kojima and Imajima 1982, Clavier 1989). The former authors found a minimum shell length of 29 mm before *H. diversicolor* became infested with *Polydora* sp. The incidence and severity of infection increased with increased abalone shell length. The trend of larger stock to become more severely affected by fouling organisms generally (Figure 6.4) has implications for the management of sea-based farms in susceptible areas. This is because certain fouling organisms have the potential to further increase mud worm settlement (see next section 6.2). The effect of stock growth on spionid settlement in subsequent years is examined in Chapter 7.

6.1.5 Conclusion

Larger stock (~50 mm) attracted greater mud worm settlement and suffered more shell damage than did stock of ~35 mm or smaller. Thus, if there were a requirement to transfer stocks to spionid susceptible sea-based sites during or soon before mud worm dispersal periods, the use of smaller animals would minimise the risk of infestation. It would be prudent to place larger abalone after the completion of the spring settlement period. Stock growth rates and cost structures for sea-based sites will determine whether individual farms use these sites for all or part of the grow-out phase.

6.2 The effect of shell fouling on mud worm settlement

6.2.1 Introduction

This section examines the effect that fouling organisms on abalone shells play in subsequent mud worm settlement. Polychaetes of the spirorbid family and *Pomatoceros* sp. were commonly observed on experimental animals after several months in the sea. Pacific oysters, barnacles, bryozoans, tunicates, coralline algae and mussels were also observed. The presence of *B. knoxi* has previously been recorded in some of these fouling organisms in New Zealand. Read (1975) noted the presence of *B. knoxi* in coralline algae on rocks and in "serpulid tube masses". These latter worms are tube-building polychaetes including members of the genus *Pomatoceros*. Handley (1997) records the presence of *B. knoxi* in *Pomatoceros* sp. in his study of the mud worm species in Pacific oysters.

Preliminary observations indicated that *B. knoxi* chimneys were often associated with the calcareous tubes secreted by spirorbids and *Pomatoceros* sp. on cultured abalone. Whereas, abalone shells coated with a distinctive red bryozoan appeared to have fewer *B. knoxi* chimneys than non-coated abalone. This was first noted by staff at Huon Aquaculture.

In this section a survey on the frequency of *B. knoxi* settlement on various abalone shell features, including fouling organisms is presented. A follow up experiment comparing the mud worm settlement rate on heavily spirorbid fouled and "clean" abalone is also described. Spirorbids were the most numerous of the fouling organisms on stock at the sea-based study sites. They were also the only fouling organism present in appreciable numbers in land-based farms observed during the study. Thus, it was considered important to determine whether the presence of these epibiont species increased the risk of mud worm settlement. A better understanding of the relationship between mud worm settlement and stock fouling may assist in the management of the risk posed by mud worms in susceptible locations.

6.2.2 Materials and Methods

Experimental animals

A survey of the location of *B. knoxi* chimneys on recently (< 6 months) infested blacklip abalone stock was conducted. Eighty animals between 20 and 40 mm, with shells relatively free of fouling organisms, were examined with low power stereo microscopy (10x). These abalone had been transferred to the sea-based study sites in August to October 1998 and 1999.

Nine abalone fouled with the red bryozoan were collected during October 1998 from Huon Aquaculture. These animals were remnant stock from a cohort that had suffered considerable mud worm mortality in 1996 and 1997. The abalone had a mean length of 56 mm (SE=3 mm) and were at least 4 years old at the time of collection. They were compared to 10 animals from the same intake time and culture vessel with out the bryozoan (length 64 ± 2 mm, $\overline{X} \pm$ SE).

Abalone for an experiment examining the effect of spirorbid fouling on spionid settlement were obtained from farm 1 on 20 July 1999, when they were approximately 18 months old. Four hundred blacklip stock, a sample of which measured 34.3 ± 0.7 mm ($\overline{X} \pm$ SE, n=200), were selected on the basis of little obvious spirorbid fouling. Subsequent microscopic examination showed mean spirorbid infestation in these "clean" stock was 7.8 ± 0.7, (n=100) per abalone. Relatively heavily spirorbid infested stock from the same size, age and tank cohort had a mean count of 62.0 ± 4.0 (n=32) spirorbids per abalone.

Experimental protocol

A survey of *B. knoxi* chimney locations was made by referring to sketches or written descriptions of chimney location made at the time of laboratory analysis. Preliminary examination of records suggested the use of common chimney location categories. All chimneys were assigned to a category, these being: on spirorbids, on other shell fouling organisms, within the apical groove of the shell or elsewhere on the shell.

In the examination of mud worm infestation rates for bryozoan covered stock, all remaining stock were collected and examined for the presence of *B. knoxi* chimneys using low power (10x) stereo microscopy. These counts were compared to counts from a sample of abalone shells from the same culture vessels with out bryozoan coatings. The null hypothesis for this survey was that there would be no difference in *B. knoxi* infestation between abalone with or without bryozoan fouling.

To test the effect of spirorbid fouling on mud worm settlement, 100 "clean" stock were placed in each of 4 basket type culture vessels as described previously (Chapter 2). Two baskets were hung within 2 m of each other at both the Aquatas and Huon Aquaculture study sites. Additionally, 11 and 23 spirorbid-infested stock were assigned to each basket at Aquatas and Huon Aquaculture, respectively. The experiments began July 21-22 1999 and were concluded mid-March and April 2000 at Aquatas and Huon Aquaculture, respectively. This exposed the abalone to one *B. knoxi* settlement period (later shown to be September 1999 to November 1999, see Chapter 3). Some nominally clean stock were removed before assessment to provide data for temporal studies on mud worm settlement: 30 at Aquatas and 100 at Huon Aquaculture.

At completion abalone were transported to the laboratory in water. Preliminary investigation had shown low rates of mud worm settlement so abalone were shucked and shells exposed to standard vermifuge solutions (Chapter 2) in replicates of five. Counts of expelled *B. knoxi* and *P. hoplura* worms were made in

replicates of five but *B. knoxi* chimney counts were performed on individual shells. Shells were assessed for mud worm shell damage by Subjective Shell Damage Rating (SSDR), (Chapter 2). The null hypothesis for this experiment was that there would be no difference in *B. knoxi* infestation between abalone with and without spirorbid fouling. Specific growth rate (Chapter 2) was calculated for length and weight in samples of recovered stock from Huon Aquaculture.

Statistical Analysis

Chimney counts of bryozoan and non-bryozoan fouled stock were compared using the Mann-Whitney *U* Test. Basket replicates in the spirorbid fouling experiment were compared using the Kolmogorov-Smirnov Two-sample test for data collected in replicates of five. The Mann-Whitney *U* Test was used for data collected from individual shells. The former test is more suitable for comparisons of small groups of data as was the case for spirorbid fouled data analysed in replicates. Where replicates were not significantly different ($P>0.05$) they were combined and further tested as appropriate between nominally clean and spirorbid fouled stock for each of: *B. knoxi* chimneys counts, *B. knoxi* and *P. hoplura* worm counts and shell damage score (SSDR). Where basket replicates could not be combined the two statistical tests described were performed separately as appropriate between "clean" and fouled stock. Figures in the results section indicate whether basket replicates were combined or not.

Specific growth rate data were compared using one tailed t-test. The SGR between spirorbid fouled and nominally clean stock were compared separately for each basket replicate. Additionally the Mann-Whitney U-Test was used for SGR (weight) comparison in one replicate, as there were too few data in one treatment group for a reliable t-test.

6.2.3 Results

Examination of recently formed *B. knoxi* chimneys on relatively clean abalone showed that settlement did not occur evenly over the shell surface but rather favoured certain sites. Although the number of spirorbids and the surface area they occupied on the abalone shell was relatively small in the examined stock, 38% of *B. knoxi* chimneys occurred on or in these fouling polychaetes (Figure 6.5). Dead spirorbid tubes were especially favoured with the chimney often projecting from the tube opening. The most commonly settled part of the abalone shell itself was the groove running around the base of the shell apex (34% of chimneys). Chimneys were also found in shell irregularities such as shell fractures, growth ridges and within respiratory pores, especially closed ones. Observation in the period 1998-2000 showed spirorbid settlement occurred in the spring and early summer in the south of the state.

As suspected bryozoan coated abalone had significantly fewer *B. knoxi* chimneys than other abalone ($P<0.01$ $U = 4.5$, Mann-Whitney U test). Means (± SE) were: 1.3 (± 0.5, n=9) and 14.2 (± 3.1, n=10), respectively. Bryozoans living on shucked abalone shells in the recirculating holding system remained alive for over 6 months but did not appear to spread nor did they colonise other nearby shells.

The quantitative spirorbid fouling experiment showed settlement of *B. knoxi* was considerably higher on spirorbid fouled stock than on "clean" stock. This was true for chimney count ($P \leq 0.01$, Mann - Whitney U Test) and surviving worm data ($P < 0.05$, Kolmogorov–Smirnov Two-sample Test) where levels were 6-10 times higher on spirorbid fouled stock at both study sites (Figures 6.6 and 6.7). Individual statistical test values are given in Appendix 6D. Note that basket replicates were not combined for the Huon Aquaculture Company site, as significant differences ($P<0.05$, Mann-Whitney U Test) existed between *B. knoxi* chimney data, *B. knoxi* worm data and SSDR for the "clean" group.

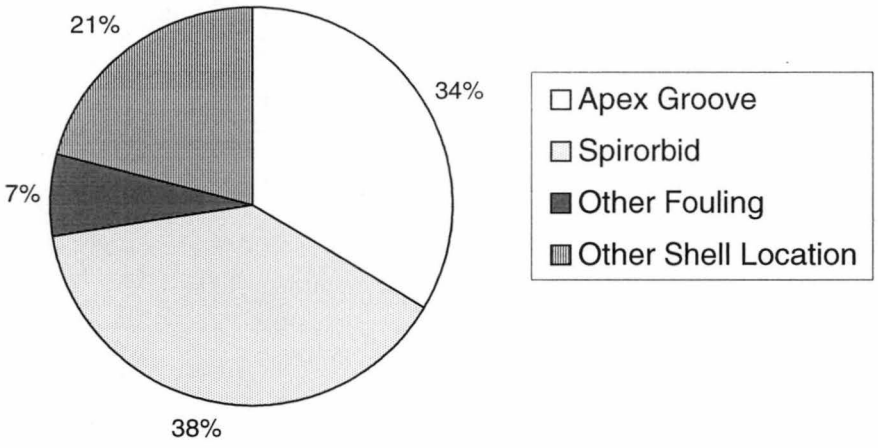


Figure 6.5. Location of *B. knoxi* chimneys (n=178 chimneys)

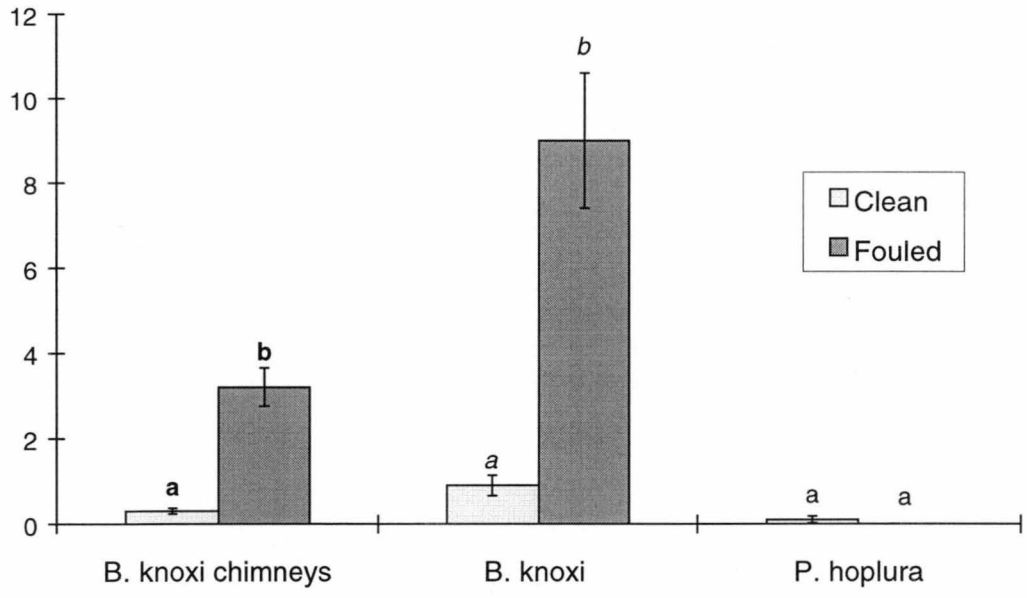


Figure 6.6. Spionid settlement in spirorbid fouled and nominally clean abalone at Aquatas. Chimney data: means ± SE n=70 and 20; clean and fouled respectively. Worm data means are replicates of 5 ± SE n=14 and 4; clean and fouled respectively. Clean and fouled data pairs with shared indicators (a, b) are not significantly different (P>0.05).

Settlement of *P. hoplura* was not as high as that of *B. knoxi* and there was no significant difference (P>0.05, Kolmogorov-Smirnov Two-sample test) between *P. hoplura* settlement on fouled and nominally clean abalone at Aquatas and the second of the Huon Aquaculture replicates (Figure 6.7). Individual statistical comparisons are given Appendix 6D.

The differences in mud worm settlement (chiefly *B. knoxi*) between spirorbid fouled and "clean" stock, led to higher levels of shell damage in the fouled stock as described by the SSDR. There was an approximately 10 fold increase in mean SSDR for fouled stock at Aquatas (P<0.01, U=125.5 Mann -Whitney U Test) and a 2- 6 fold increase at Huon Aquaculture ($P \leq 0.02$, Mann -Whitney U Test, both replicates) as compared to nominally clean stocks (Figures 6.8 and 6.9).
The differences in shell damage between spirorbid fouled and "clean" stock prompted the examination of growth between the two groups. Comparison of "clean" and spirorbid fouled stock growth rates was performed separately for each replicate (Table 6.1). Nominally clean stock grew significantly faster in replicate 1 as evidenced by SGR length (t=2.49 df 41, P<0.01, one tailed t-test) and weight (t=2.73 df 23, p<0.01, one tailed t-test). In replicate 2 only SGR weight (t = 1.80 df 36, P=0.04) indicated faster growth in nominally clean stock (SGR length replicate 2: t = 1.21 df 40, P=0.12). As the value of n was low for a reliable t-test in replicate 1, SGR weight comparison was examined using the Mann-Whitney U Test (U = 12, P=0.01). Mean growth depression as a result of increased shell blistering in spirorbid fouled stock was 28% (SD=8%, n=2) for length and 39% (SD=19%, n=2) for weight.

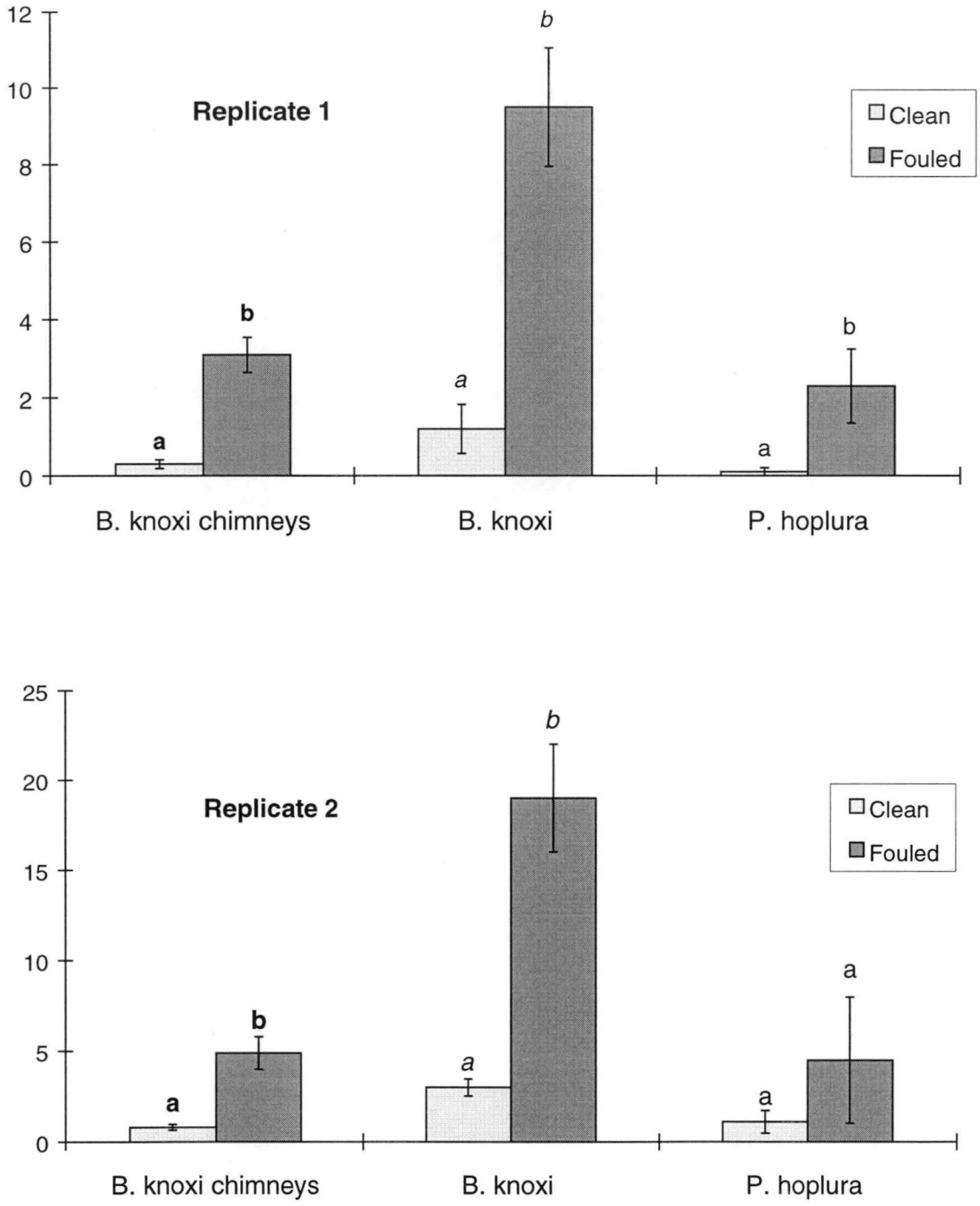


Figure 6.7 Mud worm settlement in spirorbid fouled and nominally clean abalone at Huon Aquaculture. Chimney data as mean ± SE. Replicate 1 n=50 and 20; Replicate 2 n=50 and 12, clean and fouled, respectively. Worm data means are replicates of 5 ± SE Replicate 1 n=10, 4; Replicate 2 n=10, 2 clean and fouled, respectively. Clean and fouled data pairs with shared indicators (a, b) are not significantly different (P>0.05).

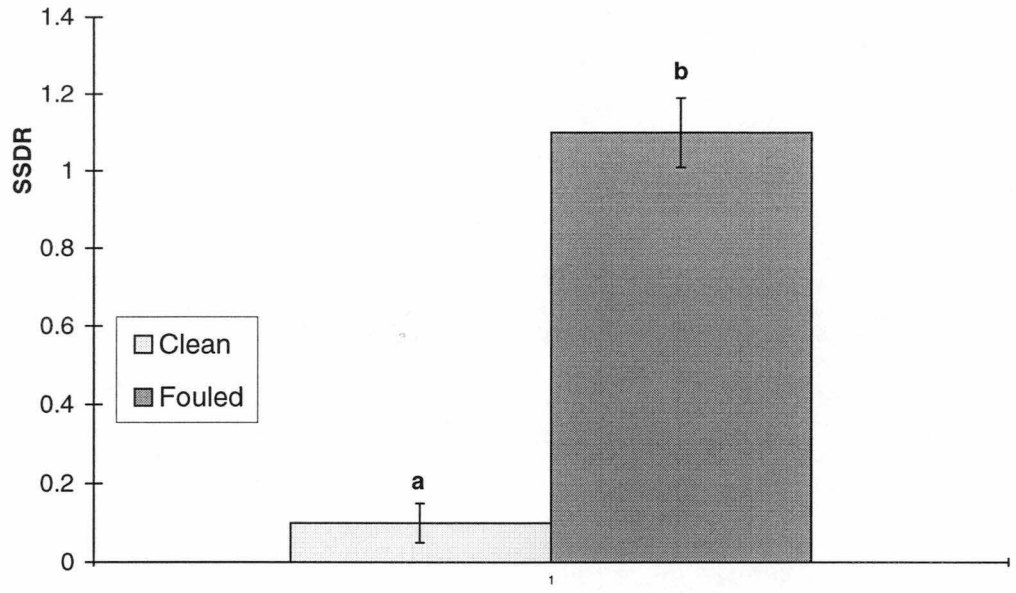


Figure 6.8 Subjective Shell Damage Ratings for nominally clean and spirorbid fouled abalone at Aquatas. Mean ± SE, n = 70 and 20 for clean and "fouled" groups, respectively. Shared indicators (a, b) signify no significant difference (P>0.05) between data pairs.

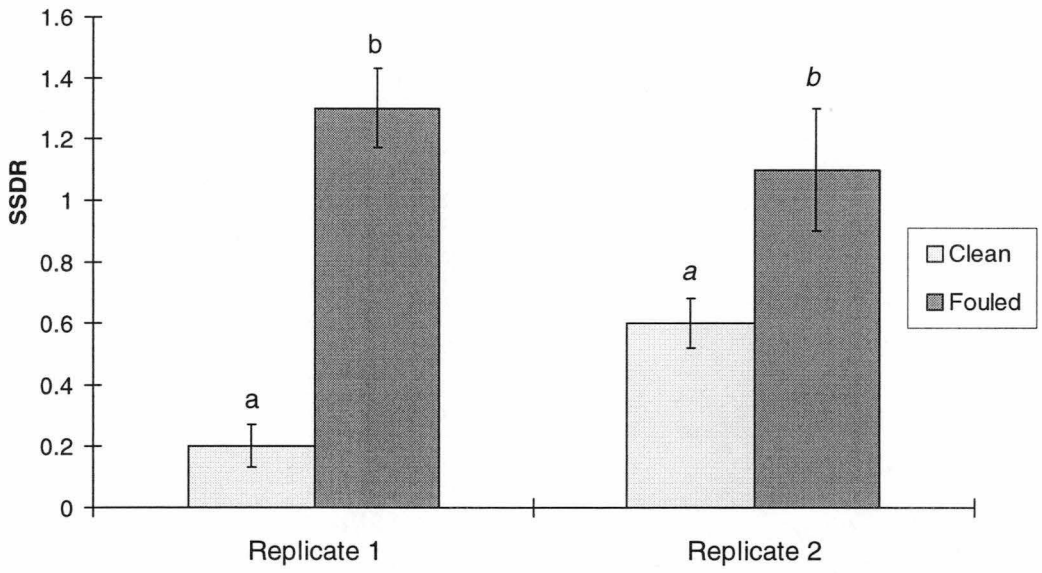


Figure 6.9 Subjective Shell Damage Ratings for nominally clean and spirorbid fouled abalone at Huon Aquaculture. Mean ± SE. Replicate 1 n = 50 and 20; Replicate 2 n = 50 and 12 for clean and "fouled" groups, respectively. Shared indicators (a, b) for replicates signify no significant difference (P>0.05) between data pairs.

Table 6.1 Comparison of growth (SGR) between "clean" and spirorbid fouled stock. Means ± SD, n in parenthesis.

	Stock status	Replicate 1	Replicate 2
SGR length	"clean"	$0.054^{A} \pm 0.020$ (24)	$0.032^{A} \pm 0.016$ (28)
	spirorbid-fouled	$0.036^{B} \pm 0.027$ (19)	$0.025^{A} \pm 0.015$ (10)
SGR weight	"clean"	$0.226^{A} \pm 0.062$ (5)	$0.133^{A} \pm 0.049$ (28)
	spirorbid-fouled	$0.108^{B} \pm 0.091$ (20)	$0.100^{B} \pm 0.055$ (10)

Data pairs by replicate with shared superscripts are not significantly different (P>0.05)

6.2.4 Discussion

Examination of *B. knoxi* chimney locations revealed that settlement was not random on the surface of the abalone shell but favoured certain areas. The apical groove was a favoured site and this was consistent with the observation that *B. knoxi* shell blisters are commonly found in the ventral shell apex region. Fouling organisms with calcareous shells such as spirorbids and, to a lesser extent, *Pomatoceros* sp. and Pacific oysters were also targeted by settling *B. knoxi* larvae. It is unclear whether larvae target these areas because they are relatively easy to burrow in or because they provide shelter (eg. the apex groove and other "shell irregularities"). Certainly the tubes of dead spirorbids may provide an instant shelter for settling *B. knoxi* larvae.

The higher rate of settlement on the spirorbid fouled relative to the "clean" group of experimental animals has implications for abalone culture in *B. knoxi*-susceptible areas. Although the extent of fouling in the spirorbid positive group was high for animals in land-based systems during the study period it was not remarkably high for abalone transferred to sea-based study sites for 3-6 months. Thus, the spirorbid fouling stock may acquire at susceptible sea-based sites predisposes them to subsequent *B. knoxi* infection. It is likely spirorbid fouling, like *B. knoxi* settlement, can be minimised by placement of stock after December of a given year. It is unclear the extent spirorbid fouling contributes to increased

P. hoplura settlement. There was little *P. hoplura* settlement at either study site and where an increase in settlement was indicated (Replicate 1, Huon Aquaculture) this was from a low base level of infection.

The settlement and subsequent increased shell blistering of the spirorbid-fouled group (Figure 6.7) led to depressed growth relative to the "clean" group (Table 6.1). Based on this result it may be cost effective to treat stock by air drying (Chapter 5) at infestation levels greater than about five worms per abalone. There is a risk treatment could itself lead to growth depression (Chapter 5, section 5.2) but at higher infestation levels the benefits probably outweigh the risks.

In a study of *B. knoxi* infestation of Pacific oysters, Handley (1997) thought it likely that induction of shell blisters incurred a metabolic cost to infested oysters. It was concluded, however, that there was no measurable significant impact on condition or growth. Examination of data presented by Handley (1997) show maximum *B. knoxi* infestation of 13.3 worms per replicate of 10 oysters when oysters were approximately 80 mm long (Table 4.1 and Figure 4.2, respectively, Handley 1997). This is a relatively low infestation as compared to the 3-5 chimneys per 40 mm abalone in this study (Figures 6.6 and 6.7). The health impacts of spionid infestation on molluscs are discussed in more detail in Chapters 7.

The bryozoan found coating abalone shells at Huon Aquaculture apparently possessed properties that reduce *B. knoxi* settlement. Unfortunately the bryozoan is relatively rare on abalone shells and appears to be slow growing. It was considered beyond the scope of this project to investigate culture potential of the bryozoan species.

6.2.5 Conclusion

Spirorbids fouling abalone shells enhanced *B. knoxi* settlement. This may pose a risk to stock when spirorbids are present in high numbers that are not uncommon at some sea-based farms. It is, therefore, prudent for abalone culture facilities in *B. knoxi* prone areas to begin grow-out with the cleanest available stock. Further, such stock should be placed after the completion of the local settlement period for spirorbids. Stock placed in December or January would not attract spirorbid settlement until the following spring and these fouling organisms would not enhance *B. knoxi* recruitment until the spring of the following year. This gives an approximate 20 month respite from the enhancement of *B. knoxi* settlement rates as a result of spirorbid fouling.

6.3 Rearing vessel design

6.3.1 Introduction

This section examines the effect of rearing container design on the progression of mud worm infestation. It was suspected that variations in water exchange as a result of design differences, particularly mesh area, could have an effect on mud worm settlement and progression. "Tube type" rearing vessels (Chapter 2, Fig 2.3A) were widely used at the time of initial spionid-related stock losses (Chapter 1) and were compared to "basket type" rearing vessels (Chapter 2, Fig 2.3B) which possess a greater mesh surface area. Any variation in mud worm infestation as a result of rearing container design would be of importance in risk assessment for sea-based grow out.

6.3.2 Materials and Method

Experimental Animals

Abalone used in the experiment were 50-65 mm, approximately 4 years old and had been spionid infested since transferral to study sites in August 1998.

Experimental Design

The study compared otherwise similar abalone stocks held in different rearing vessels in the winter/spring of 1999. Abalone were sampled before and after the *B. knoxi* settlement period comparing changes by rearing container type. At Huon Aquaculture a pair of "basket"-reared stock were compared to a pair of "tube"-reared stock. Additionally, another pair of basket-reared stock that had been treated by drying the previous December was assessed. The level of mud worm infestation in this latter basket pair was known to be lower than that of stock in the former rearing containers. This allowed comparison of trends but not of actual infestation levels.

At Aquatas one pair of "basket type" and a single "tube type" rearing vessel stocked with similarly infested abalone were available for comparison purposes. Worms were expelled from shells using chemical vermifuges (Chapter 2). Spionids were classified as "large" (> 5 mm) or small (< 5 mm) to distinguish worms likely to have settled in the previous month or two (Chapter 3, section 3.2.2). The null hypothesis was that changes in spionid numbers would not be significantly different between rearing vessels of different types. Polychaete counts before and after the *B. knoxi* settlement season and between rearing container types were compared using the Mann-Whitney *U* Test.

6.3.1 Results

Comparison of worm numbers infesting Huon Aquaculture basket-reared stock sampled before and after the *B. knoxi* settlement season showed significant increases in *B. knoxi* chimneys ($P<0.01$ $U=0.0$, Mann-Whitney U Test) and large *B. knoxi* ($P<0.01$ $U=6.5$), (Table 6.2). Count data for *P. hoplura* also increased significantly between the sample times for large worms ($P<0.01$ $U=7.0$). Non-spionid worms and total polychaetes were also significantly different between the groups ($P<0.01$ $U=17.5$ and $P<0.01$ $U=1.0$, respectively), (Table 6.2).

In contrast to data from baskets above, comparison of worm numbers infesting tube-reared stock at Huon Aquaculture showed no significant increase for *B. knoxi* chimneys ($P>0.05$ $U=39.5$) or large *B. knoxi* ($P>0.05$ $U=39$), (Table 6.3). Counts for *P. hoplura* increased significantly between the two sample times for small, large and total worms of this species ($P<0.01$ $U=7.5$, 8.5,4.5, respectively), as did counts for non-spionid worms and total polychaetes ($P<0.05$ $U=17.0$ and $P<0.01$ $U=2.0$, respectively).

Before the beginning of the *B. knoxi* settlement season at Huon Aquaculture there were no significant differences between numbers of *B. knoxi* chimneys ($P>0.05$ $U=30$), large *B. knoxi* worms ($P>0.05$ $U=42.5$), or large *P. hoplura* ($P>0.05$ $U=36$) in tubes and baskets (Tables 6.2 and 6.3).

Table 6.2 Mud worm progression for untreated Huon Aquaculture abalone reared in baskets: June 1999 - December 1999. Means ± SD

Time	*B. knoxi* Chimneys	Large *B. knoxi*	Total *B. knoxi*	Small *P. hoplura*	Large *P. hoplura*	Total *P. hoplura*	Non-spionids	Total Worms
Jun 99	$3.2^{A} \pm 1.6$	$1.7^{A} \pm 1.6$	$1.7^{A} \pm 1.6$	$0^{A} \pm 0$	$1.4^{A} \pm 1.1$	$1.4^{A} \pm 1.1$	$0.5^{A} \pm 0.5$	$4.4^{A} \pm 3.1$
Dec 99	$20.9^{B} \pm 7.7$	$10.5^{B} \pm 6.4$	$10.5^{B} \pm 6.4$	$0.3^{B} \pm 0.7$	$16.8^{B} \pm 14.5$	$17.1^{B} \pm 14.7$	$5.8^{B} \pm 8.2$	$38.2^{B} \pm 22.7$

Column means with shared superscripts are not significantly different (P>0.05)
n= 10 both data sets

Table 6.3 Mud worm progression for untreated Huon Aquaculture abalone reared in tubes: August 1999 - January 2000. Means ± SD

Time	*B. knoxi* Chimneys	Large *B. knoxi*	Total *B. knoxi*	Small *P. hoplura*	Large *P. hoplura*	Total *P. hoplura*	Non-spionids	Total Worms
Aug 99	$5.0^{A} \pm 2.9$	$2.0^{A} \pm 2.1$	$2.0^{A} \pm 2.1$	$3.0^{A} \pm 3.6$	$2.3^{A} \pm 2.4$	$5.3^{A} \pm 4.4$	$1.0^{A} \pm 2.0$	$8.9^{A} \pm 8.2$
Jan 00	$4.6^{A} \pm 3.9$	$2.3^{A} \pm 1.6$	$2.3^{A} \pm 1.6$	$12.7^{B} \pm 9.9$	$29.8^{B} \pm 23.8$	$42.5^{B} \pm 32.5$	$7.3^{B} \pm 7.5$	$52.7^{B} \pm 32.0$

Column means with shared superscripts are not significantly different (P>0.05)
n= 9 Aug 99, n = 10 January 2000 samples

Table 6.4 Mud worm progression for Huon Aquaculture abalone reared in baskets: June 1999- December 1999 (abalone previously air-dried December 1998). Means ± SD

Time	B. knoxi Chimneys	Large B. knoxi	Small P. hoplura	Large P. hoplura	Total P. hoplura	Non-spionids	Total Worms
Jun' 99	$0.2^{A} \pm 0.4$	$0.1^{A} \pm 0.3$	$0^{A} \pm 0$	$0.4^{A} \pm 0.7$	$0.4^{A} \pm 0.7$	$0.2^{A} \pm 0.4$	$0.8^{A} \pm 0.6$
Dec '99	$4.6^{B} \pm 3.6$	$3.7^{B} \pm 2.3$	$0.9^{B} \pm 1.0$	$3.9^{B} \pm 2.4$	$4.8^{B} \pm 3.0$	$1.2^{A} \pm 3.7$	$10.2^{B} \pm 4.4$

Column means with shared superscripts are not significantly different (P>0.05)
n= 9 Jun 99, n = 10 Dec 99 sample

Table 6.5 Mud worm progression in untreated tube-reared stock at Aquatas: August 1999-January 2000. Means ± SD

Time	B. knoxi Chimneys	Large B. knoxi	Small P. hoplura	Large P. hoplura	Total P. hoplura	Non-spionids	Total Worms
Aug' 99	$1.4^{A} \pm 1.3$	$1.0^{A} \pm 1.6$	$0.3^{A} \pm 0.7$	$0.4^{A} \pm 0.7$	$0.7^{A} \pm 1.1$	$6.4^{A} \pm 3.5$	$8.1^{A} \pm 4.6$
Jan '00	$1.7^{A} \pm 2.5$	$1.1^{A} \pm 1.9$	$1.9^{B} \pm 2.3$	$3.1^{B} \pm 3.0$	$5.0^{B} \pm 4.3$	$10.6^{A} \pm 9.7$	$16.7^{A} \pm 10.9$

Column means with shared superscripts are not significantly different (P>0.05)
n=10 both groups

By completion of the *B. knoxi* settlement period (sampled December for baskets and January for tubes) there were significant differences for *B. knoxi* chimneys and worms ($P<0.01$, $U=0.5$, 9.5, respectively) between baskets and tubes. In the baskets the number of *B. knoxi,* as indicated by chimney counts and recovered worms, increased approximately 5-6 fold during the exposure period, where as in the tubes there was no increase in *B. knoxi* numbers. There were no significant differences in large *P. hoplura*, total polychaetes, or non-spionid polychaetes counts ($P>0.05$ for all; $U=35.5$, 36.0, 41.0, respectively) between baskets and tubes after the settlement season. Increase in numbers of large *P. hoplura* was greater than 10 fold in both baskets and tubes.

In the less infested (previously air-dried) basket set at Huon Aquaculture the trend was the same as for the more infested basket-reared stock at the site (Table 6.2). There were significant increases in counts of: *B. knoxi* chimneys, large *B. knoxi* worms and large *P. hoplura* worms ($P<0.01$ for all; $U=2.0$, 5.5, 7.0, respectively), (Table 6.4)

As for data from the tubes at Huon Aquaculture (Table 6.3) there was no significant increase in *B. knoxi* chimneys ($P>0.05$ $U=45.5$) or worms ($p>0.05$ $U=49.5$) in tube-reared stock at Aquatas (Table 6.5). Note that there were no worms in the small *B. knoxi* category at either sample time. There were significant increases in small, large and total *P. hoplura* ($P<0.01$; $U=20.0$, 16.5, 5.5, respectively) as seen in tube reared stock at Huon Aquaculture.

Stock in the basket-type rearing vessels at Aquatas had suffered heavy mortality by early summer 2000 when they were resampled (Chapter 7). Death of abalone had been previously seen to lead to a decline in worm numbers infesting the shell (Chapter 2). Therefore, although the full range of polychaete count data had been collected in September 1999 the counts recorded in January and February 2000 for comparison purposes were considered unreliable. Data for *B. knoxi* chimney counts was considered more reliable as these structures were known to survive for several months after the death of the mud worm (Chapter 2). Chimney counts increased from 1.7 ± 1.9 (mean $\pm$ SD, n=10) in September to 3.1 ± 0.4 (mean $\pm$ SD, n=39) in January/February. This result was borderline statistically significant ($P=0.05$, $U=117.5$).

6.3.4 Discussion

The pattern of mud worm infestation over a *B. knoxi* settlement period differed between rearing container types at both study sites. Infestation levels of the mud worm species *P. hoplura* increased in both tube and basket-type rearing vessels by a factor of approximately 10. In contrast, *B. knoxi* chimney and/or worm counts increased significantly in baskets but not in tubes at both sites.

Polydora hoplura is known to produce brooded (lecithotrophic) larvae from nurse eggs. Such larvae leave the maternal burrow at a relatively large size and may omit a planktonic swimming stage (Chapter 3). On several occasions *P. hoplura* larvae were observed to form new burrows adjacent to the maternal burrow. It is plausible, therefore, that the increase in *P. hoplura* numbers in all rearing container types was substantially driven by reproduction of worms present in infested stocks.

In contrast, to the strategy of *P. hoplura*, *B. knoxi* in Tasmania appears to produce only planktonic larvae (Chapter 3). Larvae of *B. knoxi* spend 2-3 weeks in the plankton after release from capsules at a relatively small size (~ 500 μm). Therefore, by the time the larvae are ready to settle (1500-2000 μm) they may have been moved considerable distances by currents and wave action.

The smaller mesh area on tube type rearing containers (Fig 2.3A, Chapter 2) may reduce the probability of *B. knoxi* larvae from the outside gaining access to the stock, relative to the more open basket type culture vessels (Fig 2.4B). Thus, *B. knoxi* numbers increased in the more easily accessed stocks and remained static in the more sheltered stocks. Escape of hatching planktonic larvae from rearing containers may be explained as an active process since these larvae are known to be strongly phototaxic (Chapter 3). They would, therefore, be expected to migrate towards the mesh areas of the tube-type rearing containers and swim out and up towards the surface.

Fouling organisms growing on rearing containers potentially reduce the effective water exchange. Seaweed fouling on mesh was significant by summer 1999/2000 for all rearing container types. *Boccardia knoxi* mud worms were present in abalone housed in tube-type containers at the commencement of this experiment.

These worms had infested the abalone the previous spring (1998). Fouling was absent when the tube type vessels were originally placed August 1998 and was minimal by November 1998 at the end of the *B. knoxi* settlement period. Thus, mud worm settlement on abalone may depend on the interactions between reproductive strategy of the polychaete species and the effective water exchange of the containment vessel. Whether restriction of water flow in abalone rearing containers as a means of risk reduction for *B. knoxi* infestation is a worthwhile strategy is unclear. There must be a point where restriction of water flow is detrimental to abalone health. However, with respect to cleaning of rearing vessels or transfer of stock from fouled to clean containers it may be worth considering the timing of such events in relation to *B. knoxi* settlement.

6.3.4 Conclusion

The settlement intensity of *B. knoxi* was seen to vary considerably depending on the design of the abalone containment vessel. Thus, the design of rearing vessels, especially with respect to water flow, has implications for farming of abalone in mud worm susceptible areas.

Section 6.4 Spionid settlement and position in the water column

6.4.1 Introduction

This section examines the possibility of differential spionid settlement on abalone reared at different positions in the water column. Sea-based abalone farming strategies in Australia vary in respect to stock-rearing depth and in the use of cages resting on the seafloor, versus the use of rearing vessels suspended in the water column from surface structures above. Recognition of any risk factors associated with position in the water column may be a useful addition to an overall strategy for minimising mud worm impacts.

6.4.2 Materials and Methods

One hundred abalone were housed in baskets (Figure 2.3B, Chapter 2) in duplicate at each of 3, 6 and 9 m from the surface in 10 m of water at Huon Aquaculture. The 40-45 mm animals were transferred in August 2000 and exposed to spionid settlement until January 2001 (thus, including exposure to the presumptive spring 2000 *B. knoxi* settlement period - Chapter 3). Following exposure to chemical vermifuges (Chapter 2) spionid species and numbers were recorded for replicates of 10 abalone. A subjective shell damage rating (SSDR) (Chapter 2) was assigned and *B. knoxi* chimney counts were made. The null hypothesis was that there would be no difference in spionid settlement and shell damage between position treatments.

6.4.3 Results

No significant difference existed between the 6 and 9 m depth abalone for *B. knoxi* chimney and worm counts ($P>0.05$, Mann-Whitney U-test, Appendix 6E). Counts of *P. hoplura*, total spionids and SSDR were significantly different ($P<0.05$, Mann-Whitney U-test, Appendix 6.4) between the 6 and 9 m treatments (Table 6.6).

Table 6.6 Comparison of spionid settlement and impact at two positions in the water column. Means ± SE

Depth (m)	No. Chimneys	No. *B. knoxi*	No. *P. hoplura*	Total spionids	SSDR
6	$0.20^{A} \pm 0.12$	$0.15^{A} \pm 0.11$	$0.20^{A} \pm 0.12$	$0.35^{A} \pm 0.21$	$0.45^{A} \pm 0.21$
9	$0.35^{A} \pm 0.15$	$0.20^{A} \pm 0.12$	$0.95^{B} \pm 0.26$	$1.15^{B} \pm 0.30$	$1.05^{B} \pm 0.22$

Means in columns with shared superscripts are not significantly different (P>0.05)
n=10 samples for all means, each consisting of a replicate of 10 abalone

Spionid settlement was very low overall but approximately four times higher for *P. hoplura* at 9 m compared to 6 m. The 3 m depth baskets were lost before completion but an estimate of their infestation levels was available as discussed below.

6.4.4 Discussion

Spionid settlement data showed *P. hoplura* infestation was greatest 9 m from the surface, or, more importantly, 1 m from the bottom. Although, the 3 m depth treatment baskets were lost a parallel experiment (to assess timing of settlement- Chapter 3) generated monthly data for the same time period, depth and rearing vessel type. These data allowed comparison of total spionids for the period between depths but no statistical variation could be computed for the 3 m data. Mean *P. hoplura* counts at 3 m (0.35 *P. hoplura* per replicate of 10 abalone, and: 0.2 *B. knoxi* chimneys, 0.2 *B. knoxi* worms) appeared more similar to the 6 m treatment than the 9 m treatment. Greater sedimentation can be expected near the bottom and has been considered in the past to have a role in spionid infestation of oysters. Nell and Smith (1988) state that some oyster growers in NSW spray mud from oysters as this is considered to reduce the risk of mud worm infestation. Following experiments on intertidal oyster culture and mud worms, Handley and Bergquist (1997) recommended that oysters be grown at least 50 cm above the bottom to optimise spionid avoidance. Likewise Caceres-Martinez et al. (1998) found placement of oysters away from the bottom might reduce the prevalence of *Polydora* sp. infestation.

Pregenzer (1983) found that spionid infestation (which included *P. hoplura*) of the mussel *Mytilus edulis* was greatest on the bottom and in silty areas.

At some culture facilities empty shells and feral Pacific oysters may be found on the bottom and could serve as hosts for *P. hoplura.* The limited dispersal ability of *P. hoplura* may, therefore, increase the risk of colonisation in near bottom-reared stocks at some locations. By contrast, *B. knoxi* with a long planktonic larval phase (Chapter 3) has greater scope for dispersion. Recently hatched larvae were strongly phototaxic (Chapter 3) and it is plausible, therefore, that such larvae might congregate near the surface. However, *B. knoxi* settlement data in this experiment showed no significant differences at different positions in the water column. Likewise, Handley (1997) in an experiment comparing *B. knoxi* infestation of oysters at 2 depths (the surface and 6 metres) found no differences in settlement rate (generally the mean was ≤ 1 worm per replicate of ten oysters). The reason both studies found no difference between B. knoxi infestation at various depths, despite observed phototaxic larval behaviour, may be that the strong phototaxic response observed in recently hatched larvae is absent in larvae by the time they have grown to 1.5-2.0 mm and are ready to settle.

6.4.5 Conclusion

Settlement of *P. hoplura* but not *B. knoxi* was greater on abalone reared near the bottom than higher in the water column. This may have important implications for selection of culture method at some locations.

6.5 Abalone species and spionid settlement

6.5.1 Introduction

Previous observation has shown that some parts of abalone shells are favoured as sites for spionid settlement (Section 6.2). Spionids are known to settle wherever there are crevices in shell surfaces (Zotolli and Carriker 1974) and such irregularities appear to differ between greenlip and blacklip abalone and between other haliotids (Tissot 1989). The majority of abalone present at farms during previous severe mud worm outbreaks were blacklip (Chapter 1) as were most animals used in the present field research. This section examines the possibility of differential spionid settlement between blacklip and greenlip abalone, the two major culture species to date in Australia. Experimental outcomes may also be of interest in view of plans to farm *H. roei* Gray in Western Australia and *H. asinina* Linnaeus in the tropical north.

6.5.2 Method

Two hundred greenlip abalone were obtained from Tas Tiger Abalone, Dunalley (Chapter 2) at a mean length of 26.7 mm (SD = 3.2 mm). One hundred animals were placed in each of two baskets along with 100 blacklip abalone 27.3 ± 2.8 mm ($\overline{X} \pm$ SD) from farm 1 (Chapter 2).

Stock were placed at Huon Aquaculture in August 2000 and assessed January 2000, thus allowing exposure to the presumptive 2000 *B. knoxi* settlement period (Chapter 3). Abalone were shucked and exposed to chemical vermifuge solutions in replicates of ten to expel mud worms (Chapter 2). Spionids were speciated and counts compared between greenlip and blacklip abalone by use of Mann-Whitney U-test. The null hypothesis was there would not be any difference in spionid settlement between abalone species.

6.5.3 Results

Spionid settlement was very low with a maximum mean of 0.2 (SE = 0.09, n=20) *P. hoplura* per replicate of 10 abalone in the greenlip group. This was not significantly different (U=170 P>0.05, Mann-Whitney U-test) from data for the blacklip group (0.05 ± 0.05, $\overline{X}$ ± SE, n=20). No *B. knoxi* settlement was detected in either group.

6.5.4 Discussion

There is a paucity of literature on spionid preferences regarding molluscan hosts. Previous examination of wild greenlip and blacklip abalone showed that 64% of greenlip had no shell damage compared to 24% of blacklip (Chapter 1-Appendix). This shell damage was attributed at least, in part, to spionid infestation.

Previous data (Section 6.2) showed that *B. knoxi* favoured sheltered areas of the abalone shell: especially the apical groove, but also respiratory pores and shell irregularities such as fractures and growth lines. Although members of the genus *Haliotis* are morphologically very similar there are some striking differences with regard to shell sculpture and size and shape of respiratory pores (Tissot 1989). Different morphological groups have been identified and it has been suggested that these relate to ecological niches and have evolved in response to water movement over the shell (Tissot 1989). Thus, smooth shelled, streamlined species with small non-raised respiratory pores inhabit high water movement, intertidal or shallow subtidal positions in the open. Such species include *H. cracherodii* and greenlip abalone. By contrast, species with more shell sculpture and larger, raised respiratory pores (including *blacklip* abalone) inhabit more sheltered, often deeper positions (Shepherd 1973, Tissot 1989). These differences suggest blacklip may be more susceptible to spionid infestation than greenlip, as indicated by the shell damage data referred to above.

Results of the present experiment were inconclusive due to the very low rate of spionid settlement. No *B. knoxi* were recorded in the 400 abalone used in the experiment and *P. hoplura* settlement was minimal. The settlement rate recorded here must allay concerns that the high *B. knoxi* infestation previously seen at this site (Chapter 1) was a typical occurrence.

6.5.5 Conclusion

The experiment was inconclusive due to minimal spionid settlement.

Section 6.6 General discussion of risk factors

Experimental work found that *B. knoxi* settlement on abalone was not evenly distributed over the shell but favoured certain sites, including the calcareous tubes of the fouling polychaete *Spirorbis* sp. Consequently, growers in susceptible areas should attempt to minimise the spionid enhancement effect by avoiding spirorbid fouled hatchery stock in the first instance, and by placement of those animals so as to avoid natural settlement in the spring (section 6.2).

Wild abalone are rarely seen with the extent of shell fouling due to calcareous tube building polychaetes present at both southern study sites. This may suggest that the D'Entrecasteaux Channel area where these farms and others with past spionid problems are located is particularly favourable to polychaetes. Consistent with this, as noted in Chapter 1, mud worm blistering in Tasmanian oysters appears to be maximal in this area.

Two major rivers (The Derwent and The Huon) enter the sea in southern Tasmania; consequently the area is nutrient rich. Anger (1977) found that several polychaetes including the spionid *P. ligni* were useful indicators of high levels of organic pollution. This author states that *P. ligni* and some other *Polydora* sp. are associated with a high amount of organic matter and that pure sandy bottoms are normally avoided by the species. Another spionid, *Pseudopolydora reishi* Woodwick, was also associated with organically enriched areas near sewage run-off (Woodwick 1964) and two additional species *Pseudopolydora antennata* and *Pseudopolydora paucibranchiata* were also listed by the author as being associated with marine pollution. The spionid species *B. proboscidea* and *B. chilensis* were, collected by Blake and Kudenov (1981) from an area near a sewage treatment works drain in Victoria, Australia. Both these species were present in low numbers in abalone examined in the present research (Chapter 7).

Experimental work showed that position in the water column (section 6.4) might influence the extent of spionid infestation. The finding that *P. hoplura* infestation was highest near the substrate was consistent with similar studies. Pregenzer (1983) found that spionid infestation (including *P. hoplura*) of the mussel

M. edulis was greatest on the bottom and in silty areas. With regard to oysters Handley and Bergquist (1997) and Caceres-Martinez et al. (1998) recommended placement of oysters away from the bottom to reduce the prevalence of *Polydora* sp. Medcof (1945) found that percentage infestation in oysters reared off the bottom was lower than that in oysters reared on the bottom. This author also found that spionid infestation was maximised where the bottom was soft mud and salinity was low. Likewise, Caceres-Martinez (1999) found that the clam *Chione fluctifraga* Sowerby had a greater level of *Polydora* sp. infestation in an area with a muddy bottom, compared to an area with a bottom described as sandy and muddy. By contrast Dorsett (1961) reported that oysters with an accumulation of silt were less likely to suffer heavy infestations of *P. ciliata.* Dorsett (1961) found that *P. ciliata* builds a tube or chimney projecting from the host shell surface that, unlike *B. knoxi,* has sand grains embedded in it. The author showed that the spionid actively selects sand grains of a certain size. This raises the possibility that a certain sediment type might be preferred by the species.

Sinclair (1963) found that in New Zealand, *H. iris* was sometimes heavily infested with worms (up to 100) the most common of which was a spionid identified as *P. monilaris* (= *P. armata* - Read 1975). In such heavily infested shells the surface of the shell was described as lifting off exposing a network of intertwining worms and their tubes. The author also noted that shells of the species can be classified as hard or soft (the latter are unsuitable for the jewellery trade) and that soft shells come from sandy or muddy bottoms while hard shells come from clean rock or shingle bottoms. The relationship between hardness of shell and spionid infestation was not discussed. Heavily infested abalone shells are soft so it is plausible that spionid damage accounted for some of the soft shells found in sandy or muddy areas.

Environmental variables such as sediment type, wave action, temperature and salinity could not be directly tested due to limited numbers and distribution of abalone farms. Geel (1997-pers. comm.) studied abalone in Victoria, Australia and concluded that abalone from sheltered areas had higher prevalence and intensity of mud worm infestations than abalone from rough exposed waters.

He speculated that in exposed areas it was more difficult for the spionids to accumulate fine sediment to build their tubes. Anecdotal evidence from Tasmanian divers and processors suggests poor quality shells occur in sheltered areas. A shell damage assessment on shells collected previously for wild fishery research was consistent with this view (Chapter 1, Appendix 1). Baxter (1984) considered that wave action inhibiting the success of larval settlement was the most likely explanation for differential settlement of *P. ciliata* (and boring sponges) on limpets in different habitats. Similarly, Smyth (1990) in a study of bioerosion of gastropod shells in Guam, found that spionids favour sites with low surf and were absent from live gastropods in study sites with the highest surf. Clavier (1988) notes that parasite larvae are susceptible to mechanical removal just after settlement and exposure to wave action is likely to inhibit larval settlement and, thus, infestation. However, the author found no difference in levels of infestation with *Polydora* sp. between *H. tuberculata* from sheltered and exposed areas. Korringa (1951) in reviewing the impacts of spionids, especially *P. ciliata,* on Dutch oysters notes that the case of the most serious losses occurred in a sheltered area described as stagnant and that enormous numbers of worms were present on the bottom. By contrast, in places with considerable wave action and strong currents the abundance of and damage done by the spionid species was very limited.

Owen (1957) found that populations of *P. websteri* were highest in areas remote from sources of fresh water. Spionid infestation of oysters in the Mulki estuary, India was curtailed by low salinity during the monsoon season (Stephen 1978). By contrast, Medcof (1945) found *P. ciliata* in oysters was greatest in areas of low salinity in a Canadian study. Rain is potentially beneficial to spionid and host by bringing extra nutrients into the environment from run off as noted above. In contrast, large decreases in salinity are potentially harmful to spionids (Chapter 4), whereas bivalves may be able to survive such conditions by closure of their shells. In view of the literature on shelter and salinity it is interesting that of the longer standing Tasmanian farms, the site with the highest energy coastline, furthest from an estuary has had no incidence of spionids.

Smyth (1989) found that mollusc shells coated with crustose coralline algae had fewer spionid burrows than similar non-coated shells. By contrast, Read (1975) and Handley (2000) found that *B. knoxi* bores into coralline algae encrusting molluscs (including *H. iris*) and rocks. Similarly, *B. knoxi* in Tasmania has been found in wild *H. rubra* with a coralline algal coat.

In addition to the degree of spirorbid fouling and size of animals at transfer (section 6.1), position in the water column is under the control of the culturist and may be useful in minimising spionid impacts. It is unclear whether the finding of variable spionid settlement on abalone reared in different vessels (section 6.3) can be exploited by farmers. The inconclusive comparison of spionid settlement between abalone species (section 6.5) may require repeating in the future if the industry expands its production, farm sites and culture species.

Certain environmental variables not directly tested but referred to above may be important at the site selection stage of an abalone culture venture. In summary it would appear to minimise risk of spionid infestation abalone farms should not be located in very sheltered areas with soft, muddy or silty substrates. Estuaries and sources of run-off, including organically enriched water should also be avoided. Dinamani (1986) suggests that large aggregations of oysters may attract spionids and therefore populations of wild oysters should also be avoided.

ventral view - farmed greenlip, raised blisters - P. hoplard?

Frontispiece: abalone shell with mud worm blisters

Chapter 7
GROWTH, MORTALITY AND SHELL DAMAGE

7.1 Introduction

Spionid mud worms in wild abalone species have been described by Sinclair (1963: *H. iris* in New Zealand), Hansen (1970: *H. rufescens* and *H. cracherodii* in California), Horne (1996: *H. kamtschatkana*, Canada), Kojima and Imajima (1982: *H. diversicolor*, Japan) and Clavier (1989: *H. tuberculata*, Channel Islands - Europe). The latter two studies included aspects relating to abalone health. There is, however, a paucity of reports in the literature concerning spionid infestation in cultured abalone. The present research was initiated in response to spionid infestations and stock mortality at a number of Tasmanian facilities in the mid to late 1990's (Chapter 1). In recent times, reports of infestations of farmed stock in other locations have been communicated. These include infestations of: *H. discus hannai* in Chile (McCormick 1999, pers. comm.), *H. kamtschatkana* brood stock in Canada (Biagi 2000, pers. comm.), *H. laevigata* in Western Australia (Freeman 2000, pers. comm.) and *H. iris* in New Zealand (Handley 2001, pers. comm.). Mortality was associated with stocks in Chile and Western Australia.

Growth depression and mortality have been reported in cultured South African *H. midae* abalone infested with a sabellid polychaete rather than spionid polychaete (Ruck and Cook, 1998). Like a spionid infestation, the sabellid infestation results in disruption to normal shell structure, but without chemical dissolution of the shell (Culver et al. 1996)

The present study describes the affects of spionids on farmed abalone health in field trials between 1998-2001. Identification of levels of spionid infestation that have significant outcomes for industry, such as stock mortality and growth depression, will assist farmers in minimising the economic impact of mud worm. Reporting on the health implications of mud worm infestations are split between

two chapters. This chapter deals with the more basic measures such as mortality, growth and condition indices while Chapter 8 reports on physiological and histological changes.

7.2 Methods

7.2.1 Experimental Animals

Abalone used in health studies were originally from farm 1 (Chapter 2) and had been transferred to study sites in August - December 1998 and 1999, initially as part of spionid settlement timing experiments (Chapter 3). The August 1998 transfer cohort were older and somewhat larger than other stocks (Chapter 2.1) and because of subsequent infestation patterns are distinguished from other abalone in the results section.

As spirorbid fouling was found to have implications for subsequent mud worm settlement (Chapter 6.2) retrospective counts were performed on shells from the August and September-November 1998 intakes which had been sampled before the end of 1998. Median fouling with spirorbids larger than 3 mm (and therefore, considered potential targets for spionid settlement) was three per abalone for the August 1998 intake stock (mean=6.0, SD=8.4, n=70 shells). The same number of shells from the September-November 1998 sample was found to have a total of two spirorbids.

7.2.2 Holding conditions and study sites

The study sites in southern Tasmania were at the Aquatas and Huon Aquaculture Company facilities with another study site off the east coast belonging to Tasmanian Scallops (location maps, Chapter 2). All stock transfers to the southern study sites consisted of 100 abalone in at least two replicates. Abalone transferred to the study sites in August and spring 1998 were housed initially in

"tube type" rearing vessels (Chapter 2). Stocks placed in the period September-November were consolidated into "tray type" containers in March or April of the following year and are referred to as the spring 1998 intake in the results section. This process was repeated in 1999 (spring 1999 intake), except abalone were initially housed in baskets (Chapter 2) instead of "tubes". Unless otherwise stated August 1998 transferred abalone remained in tubes throughout the study period. Abalone were fed as described in Chapter 2.3. Abalone were not transferred to the Tasmanian Scallops site specifically for study purposes; rather samples of pre-existing commercial stock were made available for study (Chapter 3.2.2)

7.2.3 Blister morphology

The size and severity of mud worm blisters was assessed by the blister tracing and subjective shell damage rating methods as described in Chapter 2. The location of blisters was quantified by assigning blisters to a type locality map as depicted in Figure 7.1. Six hundred blistered shells were assessed and separate location records kept for those shells infested by a single species of spionid.

The volumes of a sample of the largest mud worm blisters encountered were measured by allowing shells to dry, puncturing the blister and then submerging shells in water. The water was then removed from the blister with a 21 gauge needle and 1-5 ml syringe as appropriate and the volume recorded. The volume of large blisters in abalone was compared to a sample of large blisters formed in Pacific oysters by *B. knoxi* at Huon Aquaculture. Histological examination of blisters was performed on seawater formalin fixed, decalcified shells. Routine processing was performed as described Chapter 2.

7.2.4 Spionid quantification

Spionid mud worms were expelled from their burrows by the vermifuge method (Chapter 2), counted and classified by species and by size. Mud worms less than 5 mm were classed as "small" and those above 5 mm as "large". This was done to allow the larger worms, which contributed most if not all of the blistering, to be distinguished from the total set of mud worms.

7.2.5 Measures of abalone condition

The condition or relative "fleshyness" of abalone was assessed by calculating the percentage flesh weight. This is simply the shucked flesh weight divided by the whole live weight and multiplied by 100. An advantage of this method was that after measurement the flesh could be fixed in formalin for histology or frozen for later chemical testing. Dry weight indices were calculated by separating meat and shells and drying in an incubator at 70° C for 48 h until constant weight was achieved.
The dry weight index CI_{LENGTH} was calculated as:

$$CI_{LENGTH} = \frac{\text{Dry flesh weight (g)} \times 100}{\text{Shell length (mm)}}$$

The dry weight index CI_{WEIGHT} as recommended by Lucas and Beninger (1985) was calculated as:

$$CI_{WEIGHT} = \frac{\text{Dry flesh weight (g)} \times 10}{\text{Dry shell weight (g)}}$$

The dry to wet flesh weight ratio as also recommended by Lucas and Beninger (1985) for use in aquaculture studies was also calculated. The various indices were compared using non-infested abalone to find the most suitable measure for long-term health assessment.

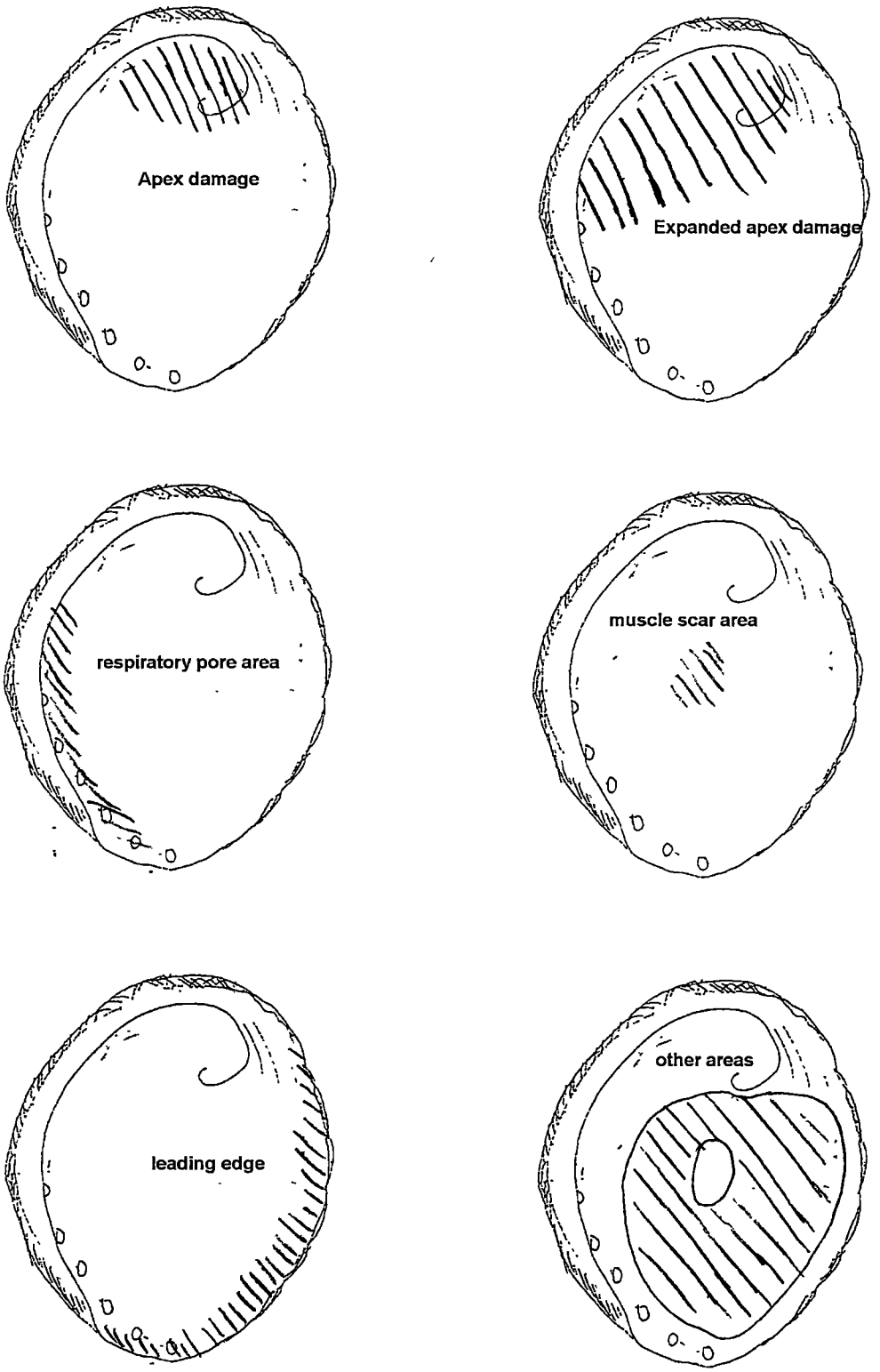


Figure 7.1 Common mud worm blister locations in abalone

7.2.6 Experimental design

Placement of mud worm free stock at two sea lease sites with a history of severe mud worm infestation was intended to recreate the conditions which led to stock mortality in the mid to late 1990's. The hatchery, feed source, and containment vessels used during previous infestation periods were also used throughout this study. Periodic sampling was performed to determine spionid infestation levels and the consequences of infestation in terms of stock mortality and health indicators such as growth, condition and shell blistering was quantified.

By allowing a stock cohort to become infested and then treating a portion of that stock to eliminate or reduce spionid infestation, the affects of the infestation were compared for otherwise matched groups. The null hypothesis was that differential spionid infestation levels would have no affect on abalone health measures including mortality rate, growth, shell blistering and condition indices. This experiment was conducted using August 1998 intake abalone at Huon Aquaculture and stock assigned to this experiment are referred to as "cohort 1" in the results and discussion sections.

To control mud worm infestation levels in one group of stock relative to the other, air drying of half the animals was conducted in December 1998 and 1999. Details of the drying conditions and efficacy are given in Chapter 5 (Trials 6 and 10). December was chosen as the drying period as initial data suggested that larval settlement of *B. knoxi*, the major focus of the research, had concluded by then (Chapter 3). For the experiment comparing the health of matched infested and non-infested stock to succeed, long-term suppression of spionid infestation in one of the groups would be necessary. This was an experiment in itself: with the null hypothesis that annual post *B. knoxi* settlement season air drying treatment would not affect infestation by this species.

The recommendation from Chapter 3 that *B. knoxi* infestation could be avoided by placement of stock post November was tested by comparison of stocks placed in September 1999 and December 1999 at both southern study sites.

The null hypothesis was that there would be no difference in *B. knoxi* counts and shell blistering between the two intake time cohorts.

7.2.7 Statistical methods

Long-term treatment trial data (Huon Aquaculture August 1998 cohort 1) were analysed using ANOVA or Residual Maximum Likelyhood (REML) in situations where sample sizes varied giving an unbalanced non-orthogonal design. Thus, spionid count data were analysed using two-way ANOVA, after log transformation of data; blister coverage data was analysed using REML. Percentage flesh weight data was arcsine transformed prior to analysis using 2 way ANOVA. The non-parametric Mann-Whitney U Test was used to analyse SSDR data. Linear Regression data were analysed using the simple linear regression option in Genstat. Residual plots were examined to check for major departures from the assumptions of normal distribution and uniform variance.

7.3 Results

7.3.1 Mortality

Generally, there was little mortality that could be attributed to spionid mud worm infestation. Approximately 9000 abalone were transferred to the study sites for experimental work detailed in Chapters 3, 6 and the present chapter. Of these, only a few hundred animals became heavily mud worm infested. The two most heavily infested cohorts provided a contrast in mortality data.

At Aquatas the heavily infested August 1998 intake group experienced mortality of 48 of 61 (79%) remaining abalone between November 1999 and June 2000. Whereas, in the similarly infested, same intake time cohort at Huon Aquaculture there were three abalone deaths (from initial pool of 200) between May 1999 and Feb 2000 featuring severe blistering. In the minimally mud worm infested

spring 1998 intake at Huon Aquaculture there was a 7% mortality rate over the length of the study.

Mortality rates of up to 30% were experienced in some stock groups in the spring of 1999 and 2000 within the first 2 months following transfer from the source farm to Huon Aquaculture. Possible reasons for this are given in the discussion section.

7.3.2 Growth

Growth was generally poor by commercial abalone culture standards as shown in Table 7.1. The August 1998 intake at Aquatas that experienced considerable mortality showed a mean reduction in weight over the course of the study. Growth data is presented in more detail below for those groups in which a significant degree of mud worm infestation existed.

August 1998 Cohort 1 Huon Aquaculture

Growth comparison between mud worm infested stock and the matched group treated by drying to reduce infestation levels is shown in Figure 7.2. At the time of treatment (December 1998) there was no significant difference in length of the two groups but by July 1999 the air dried group were significantly longer ($P<0.01$, $t=-2.79$, df 152, two-tailed t-test). Based on group mean comparisons treated stock grew at 57 microns.day $^{-1}$ compared to 44 microns.day $^{-1}$ for untreated stock. This is a growth depression of approximately 20% attributable to the level of mud worm infestation present in the time period . As seen in Figure 7.2 growth of both treated and untreated abalone was poor in this intake cohort after July 1999. Between July 1999 and October 2000 both groups grew only another 3 mm, which corresponded with a reduction in feeding frequency from approximately weekly to twice monthly.

August 1998 Cohort 2 Huon Aquaculture

This group was reared in "tube type" vessels throughout the length of the study. As seen later, this group was subject to very high levels of mud worm infestation including shell blistering and grew from approximately 46 to 67 mm in over 2 years (Table 7.1).

Table 7.1 Size and growth data for abalone at three farm sites, mean ± SD (n)

	Huon Aquaculture Company					
cohort	Aug 1998 (1)	Aug 1998 (2)	Spring 1998	Spring 1999	Dec 1999	Apr 2000
Date in	11/8/98	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	10/10/00	21/11/00	9/1/01	11/11/00	9/1/01	9/1/01
Time (d)	791	833	843	473	392	274
Initial length (mm)	46.9 ± 2.6(100)	46.2 ± 3.1 (100)	18.8 ± 1.4 (150)	42.0 ± 6.1 (100)	28.2 ± 3.2 (100)	31.5 ± 4.3 (172)
Initial weight (g)	15.4 ± 2.8 (100)	14.7 ± 2.9 (100)	1.0 ± 0.2 (150)	10.7 ± 4.5 (100)	2.9 ± 1.0 (100)	4.2 ± 1.8 (172)
Final length (mm)	61.8 ± 5.5 (23)	66.9 ± 7.1 (49)	57.6 ± 6.0 (94)	43.4 ± 4.7 (10)	42.6 ± 5.3 (10)	44.7 ± 5.0 (10)
Final weight (g)	36.0 ± 10.1 (23)	46.7 ± 13.0 (49)	25.0 ± 11.4 (88)	12.2 ± 3.9 (10)	11.9 ± 5.4 (10)	13.4 ± 3.9 (10)
Growth: length ($\mu.d^{-1}$)	18.8	24.9	46.0	3.2	36.7	48.3
Growth:weight ($mg.d^{-1}$)	26.1	38.4	28.5	3.5	22.9	33.7
SGR length	0.035	0.045	0.133	0.008	0.105	0.128
SGR weight	0.108	0.139	0.383	0.031	0.361	0.427

Huon Aquaculture August 1998 intakes: (1) = control group from long term mud worm treatment experiment, (2) = stock reared in "tube" type containers

	Aquatas				Tasmanian Scallops
cohort	Aug 1998	Spring 1998	Spring 1999	Dec 1999	April 2000
Date in	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	13/6/00	12/11/00	10/10/00	11/4/00	29/11/00
Time (d)	671	793	382	119	278
Initial length (mm)	46.9 ± 2.7 (200)	20.9 ± 2.1 (50)	39.1 ± 4.3 (100)	26.1 ± 3.6 (100)	33.8 ± 4.6 (10)
Initial weight (g)	15.7 ± 2.8 (200)	1.2 ± 0.4 (50)	8.5 ± 2.7 (100)	2.4 ± 1.0 (100)	9.5 ± 3.7 (10) *
Final length (mm)	47.6 ± 2.5 (10)	34.5 ± 4.4 (18)	40.3 ± 3.6 (10)	27.5 ± 3.7 (20)	50.4 ± 6.8 (10)
Final weight (g)	12.8 ± 2.7 (10)	5.5 ± 1.7 (18)	9.2 ± 2.9 (10)	2.7 ± 1.1 (20)	20.5 ± 8.1 (10)
Growth: length ($\mu.d^{-1}$)	1.0	17.1	3.0	12.0	71.6
Growth:weight ($mg.d^{-1}$)	-4.4	5.3	1.8	3.1	62.3*
SGR length	0.002	0.063	0.008	0.045	0.172
SGR weight	-0.031	0.186	0.020	0.122	0.435*

* = First Tasmanian Scallops weight data taken June 6 2000, later than first length data.

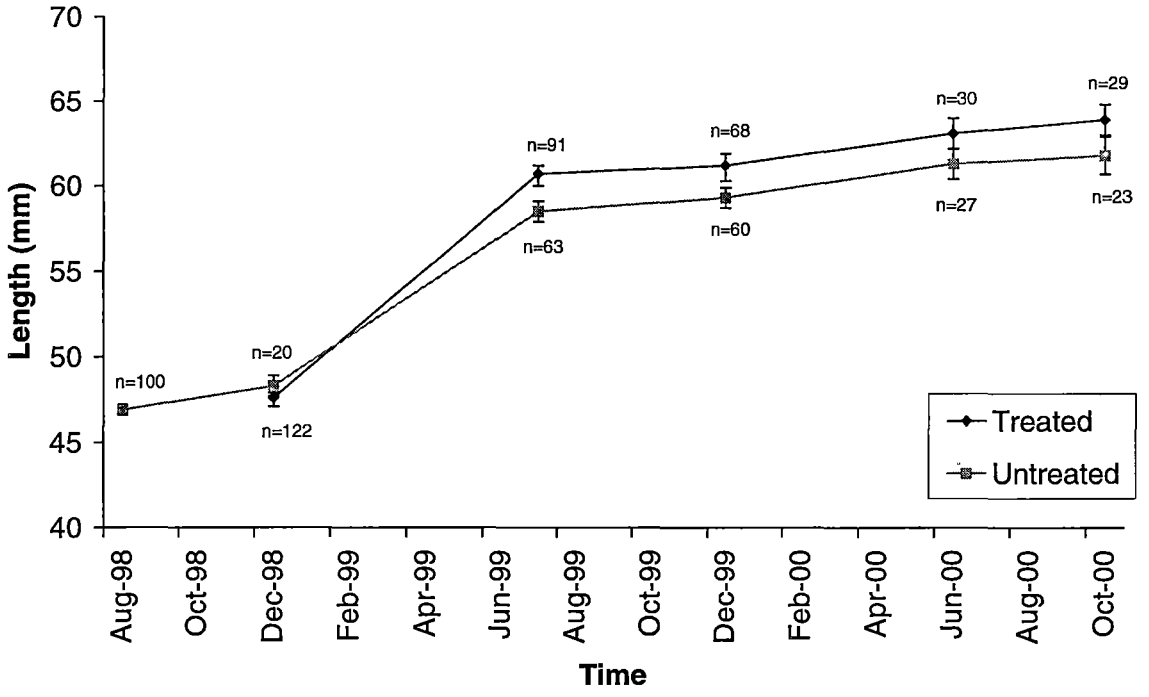


Figure 7.2 Length comparison for mud worm treated and untreated abalone. Huon Aquaculture August 1998 intake (1). Means ± SE.

Growth of other abalone intake time cohorts

The spring 1998 cohort at Huon Aquaculture Company was stocked at approximately 20 mm or less and approached market size (~60 mm) about 2 years later in early January 2001 (Table 7.1). Specific growth rates (Table 7.1) were similar for the spring 1998, December 1999 and April 2000 cohorts at Huon Aquaculture

Poor growth of stock at Aquatas generally was linked to increasingly infrequent feeding in the second half of the project. Stock sampled at Tasmanian Scallops grew faster than stocks at the other study sites (Table 7.1). All 3 sites used the same sources for spat and feed. Aquatech® trays were used to grow the stock at Tasmanian Scallops and for some intake cohorts at Aquatas and Huon Aquaculture.

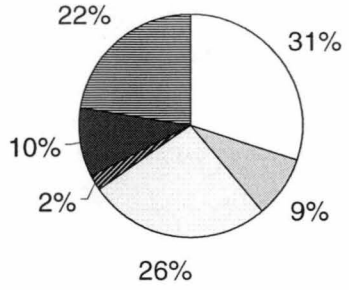
7.3.3 Shell blistering and spionid counts

Shell blistering was most common in the apex area of the shell, with blisters in the vicinity of the respiratory pores the second most common. (Figure 7.3A). Blisters caused by *B. knoxi* worms were located overwhelmingly in the shell apex area (63% Figure 7.3B) contrasting with *P. hoplura* blisters that tended to be more evenly spread around the host shell (Figure 7.3C).

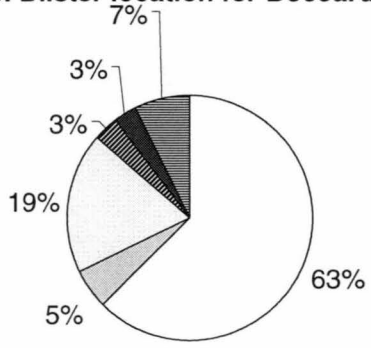
Early mud worm blisters were flat and yellow in colour (Figure 7.4) a result of initial conchiolin deposition (Blake and Evans, 1972). Actively growing *B. knoxi* blisters tended to be amber to light brown in colour and were frequently located in the shell apex (Figure 7.5) as indicated by Figure 7.3B. Eventually such blisters usually developed a thick coat of nacre over them (Figure 7.6) that contrasted with the soft, non-healed blisters associated with the initial mortality reports.

The variability of blister location with *P. hoplura* infestation is shown in Figures 7.7-7.9, including damage to the leading edge, respiratory pores, apex and other areas. With time *P. hoplura* blisters became dark brown/ black in appearance

A. Blister locations, mixed spionid infestation



B. Blister location for *Boccardia knoxi*



C. Blister location for *Polydora hoplura*

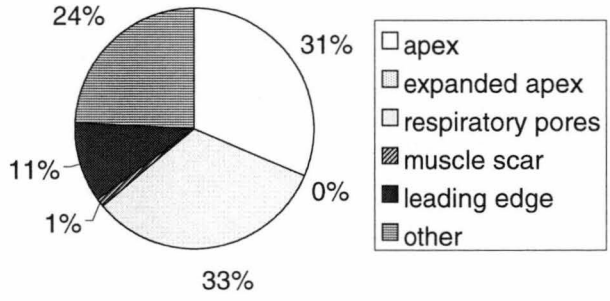


Figure 7.3 Locations of shell blisters for spionid infested abalone (n=600, 81 and 70 shells for A, B and C respectively)

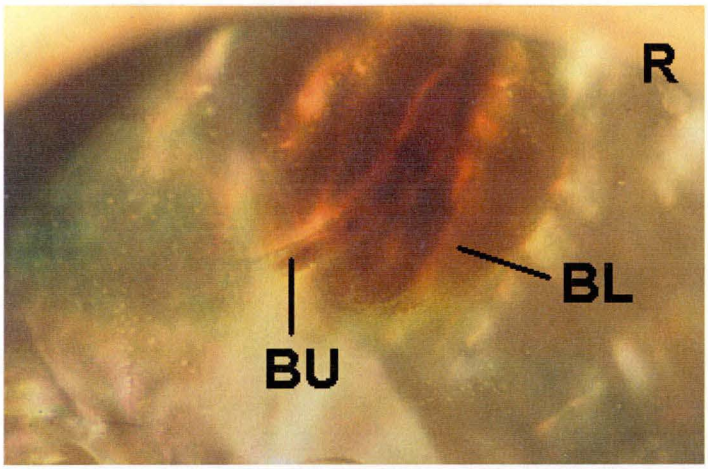


Figure 7.4 Early blister in shell apex. BL = blister, BU = spionid burrow, R = shell rim

Figure 7.5 Early *Boccardia knoxi* blister. A = shell apex

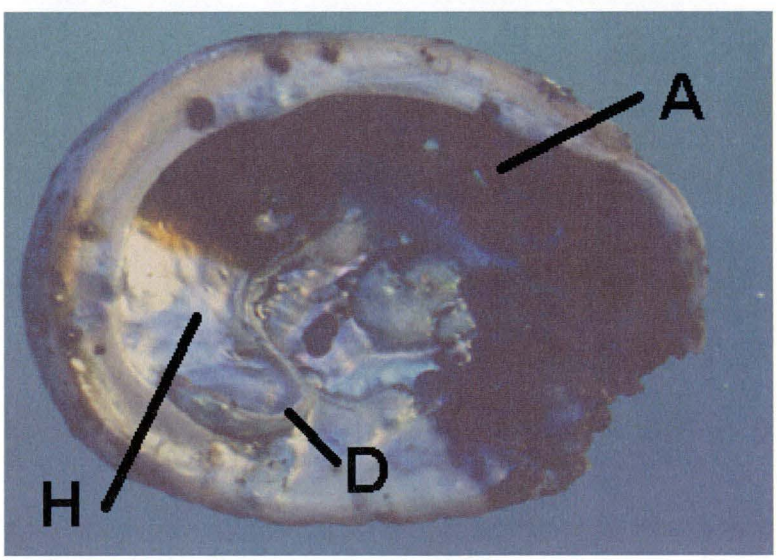


Figure 7.6 Severe mud worm infestation. A = active *P. hoplura* blister, H = healed *B. knoxi* blister, D = shell deformity around conical appendage

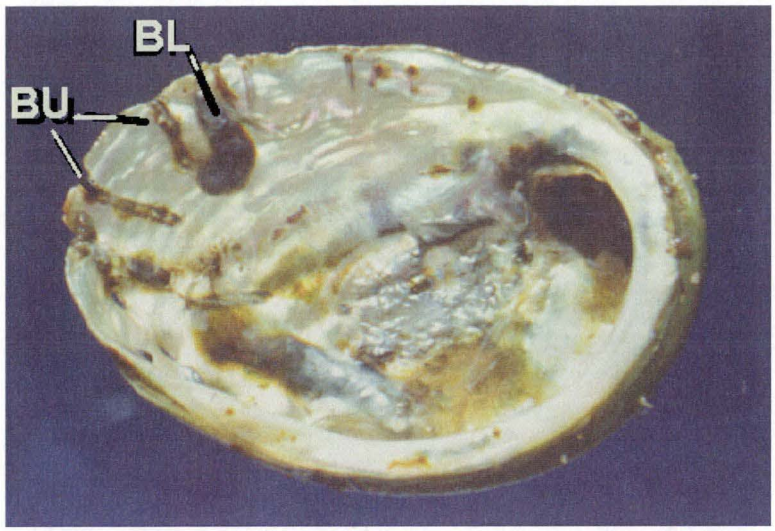


Figure 7.7 *P. hoplura* infestation at leading edge of shell. BL = blister, BU = burrows

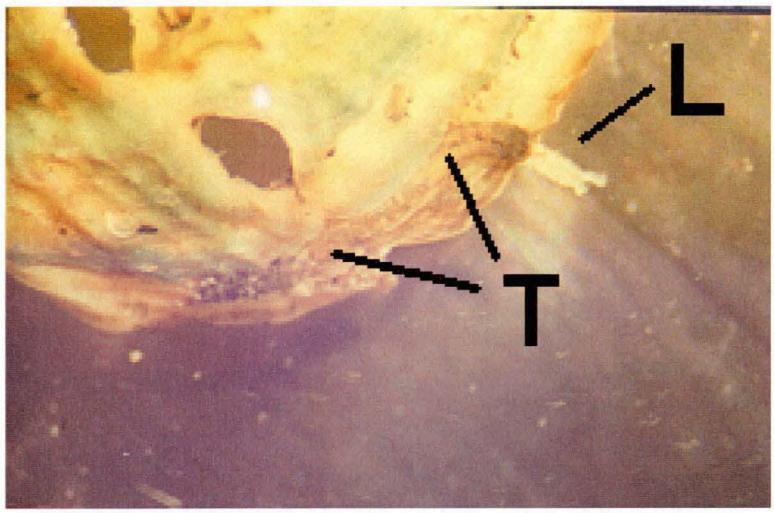


Figure 7.8 *P. hoplura* larva (2-3 mm) at shell edge. L = larvae, T = tube

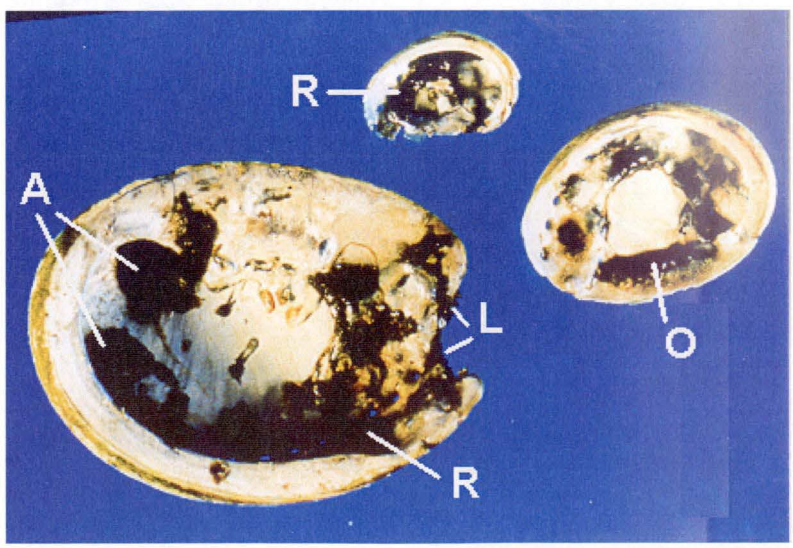


Figure 7.9 Locations of *P. hoplura* infestation. A = apex area, L = leading edge, O = "other " area, R = respiratory pore area

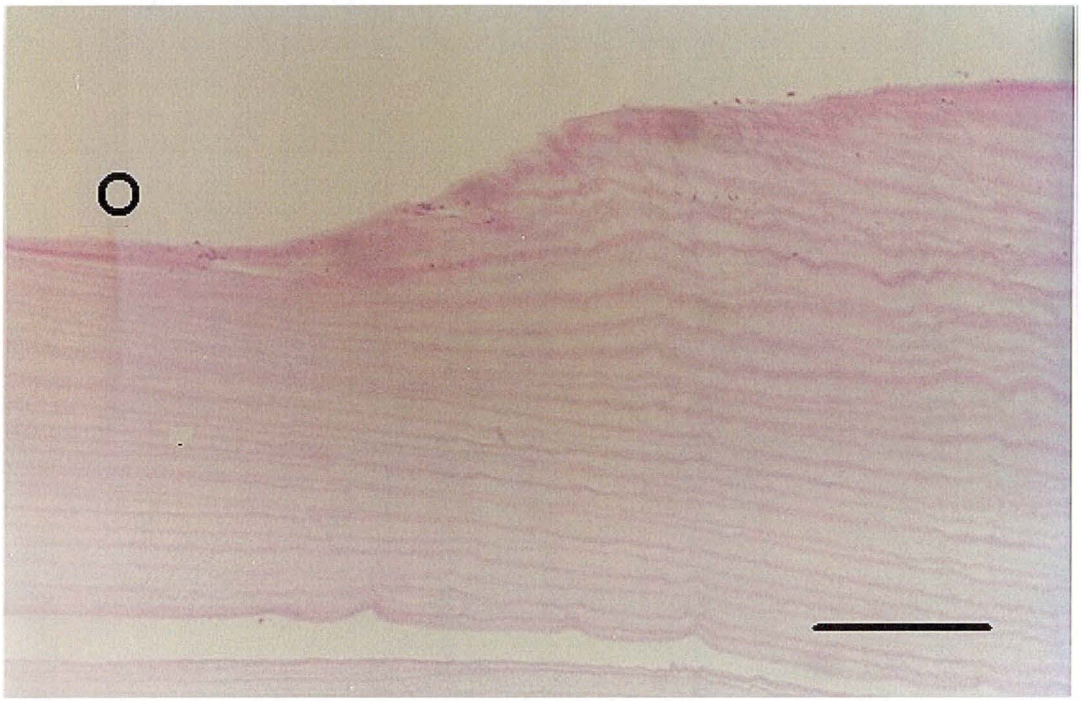


Figure 7.10 Normal abalone shell structure, O = outside of shell, Bar = 100 μm, H & E stain.

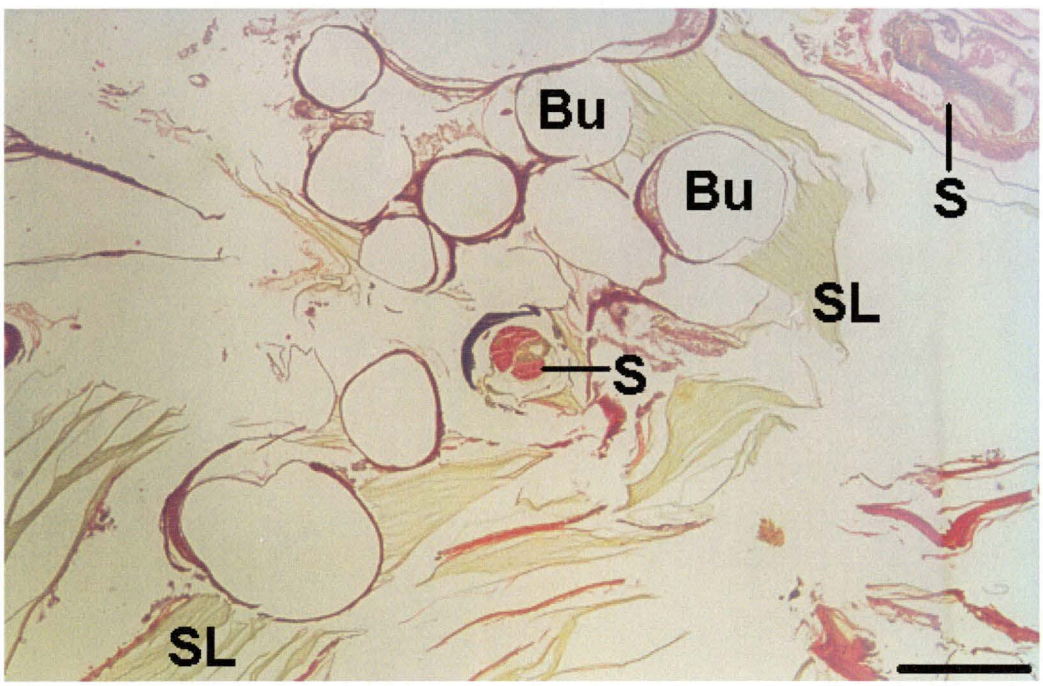


Figure 7.11 Cross-section of abalone shell honey combed with spionid burrows. SL = disrupted shell layers, BU = spionid burrows, S = spionid in cross-section. Bar = 500 μm, PAS stain.

(Figure 7.9) unless substantial shell nacre was deposited over them. Older blisters of both spionid species were raised up to 5 mm from the base of the shell. Long-term infested abalone usually had a mixture of spionid species and corresponding damage to the shell. Figure 7.6 shows an abalone with a healed, albeit deformed shell apex caused by *B. knoxi* and substantial damage to the respiratory pore area and leading edge caused by poorly healed *P. hoplura* blisters. Three additional species of spionids were found in abalone shells infrequently. These were: *B. chilensis*, *B. proboscidea* and *P. armata*.

The volumes of 10 representative large blisters from the apex area of the shell were measured. These blisters, caused by *B. knoxi* and had volume range of 0.1 to 0.6 ml. By contrast the volume of a sample of large *B. knoxi* blisters measured in Pacific oysters was an order of magnitude greater, with one oyster recording a volume of 10 ml. Histological cross sections of shell, including blisters, mud worms and blister detritus are shown in Figures 7.10 and 7.11.

August 1998 cohorts

In cohort 1 at Huon Aquaculture shell blistering was detected September 1998, one month after transfer to the site (Figure 7.12). "Active" category blisters (Chapter 2) dominated samples for the first few months but by 1999 blisters assigned to the "healed" category were common, indicating nacre was being deposited. By October 2000 shell blistering in the untreated control stock had reached approximately 30% of shell area (Figure 7.12). This was comparable blister coverage to that associated with the initial abalone mortality episodes of the mid-1990's (Chapter 1). Comparison of percent total blister coverage in treated stock (air dried in December 1998 and 1999) and control untreated abalone is shown in Figure 7.13 REML analysis with time and treatment as factors was highly significant ($P<0.001$) for both factors (Refer to Appendix 7A). Untreated stock were significantly more blistered than treated stock from the same time sample in March and October 2000 (Figure 7.13).

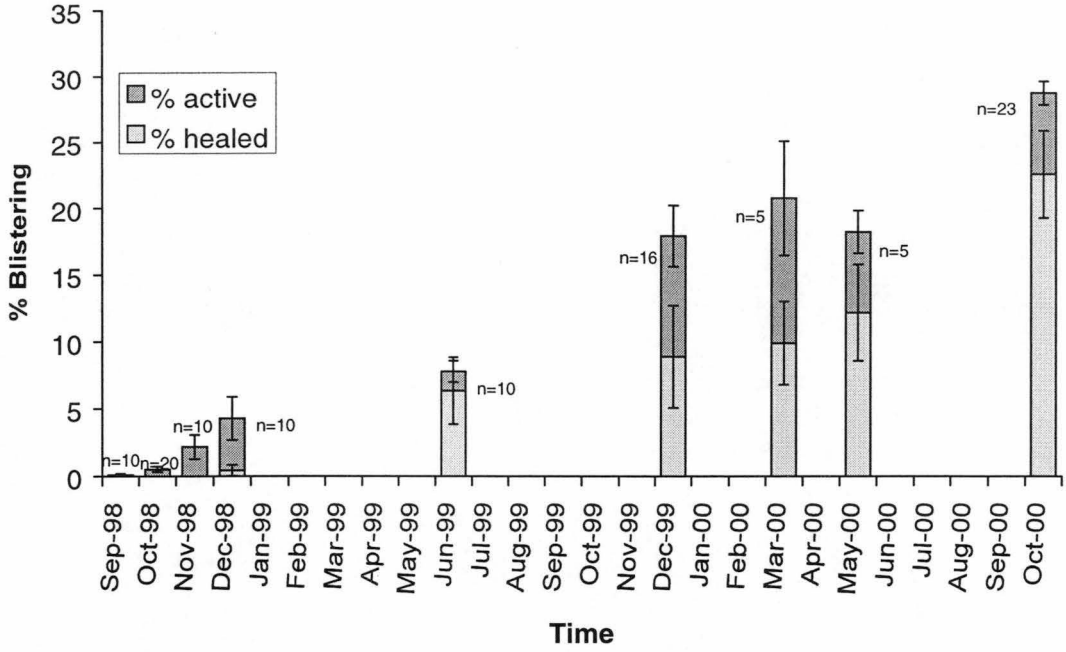


Figure 7.12 Progression and nature of shell blistering Huon Aquaculture August Intake cohort 1 (untreated control abalone) means ± SE

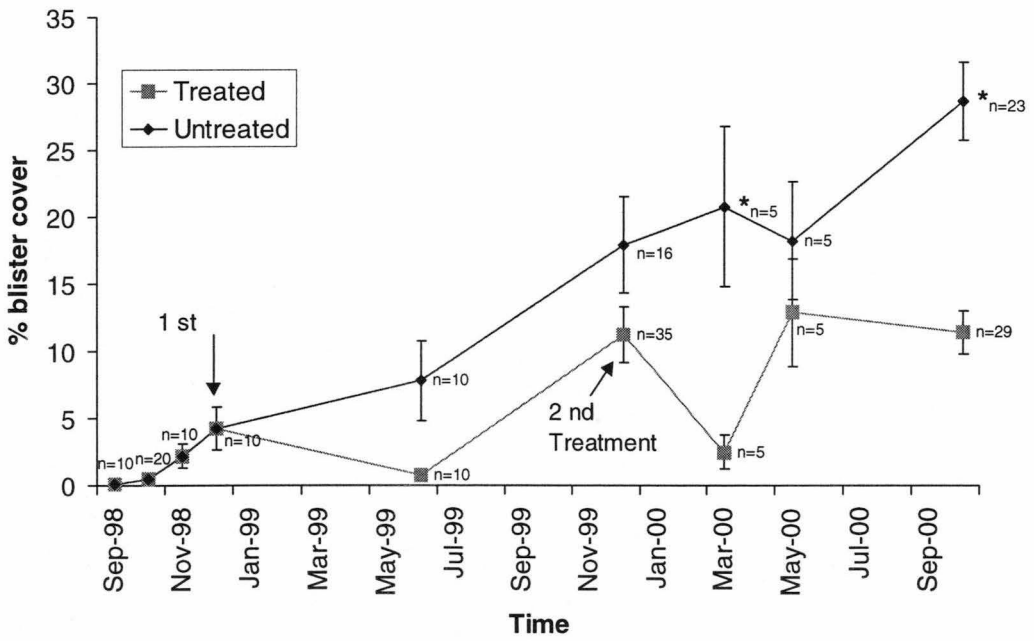


Figure 7.13 Mud worm blister comparison for treated and untreated abalone Huon aquaculture August 1998 intake (means ± SE). * denotes significantly different data Pairs (P<0.05).

Subjective blister data showed the maximum SSDR value of "3" was recorded in samples from untreated stock in 2000 but not in previous years (Figure 7.14). The treated abalone recorded fewer Grade 3 SSDR scores and contrasting with the untreated stock recorded some zero SSDR scores during 2000 (Figure 7.14). Mann-Whitney U Test analysis of SSDR data for October 2000 (final sample) showed a significant difference between the treated and untreated stock ($P<0.01$, $U=196$).

Spionids were present in abalone shells within a month of transfer and mean counts were approximately 50 per shell by May 2000 (Figure 7.15). This level was previously associated with stock mortality (Chapter 1). However, a dominance of *B. knoxi* worms were previously recorded in the shells of dead and dying abalone at the site, contrasting with this study in which *P. hoplura* dominated numerically (Figure 7.15). Air drying treatment (December 1998 and 1999) significantly reduced mud worm counts over time (Figure 7.15). Two-way ANOVA performed on log transformed data with time and treatment as factors was highly significant ($P<0.001$ for both factors and for both spionid species). Refer to Appendices 7B and 7C for ANOVA table. Counts of *B. knoxi* in untreated stock were approximately five times higher than in air dried stock over the duration of the study. This difference was statistically significant at every time sample ($P<0.05$). Similarly, counts of *P. hoplura* were significantly ($P<0.05$) higher in untreated stock at all sample times except June1999. Spionids of both species increased markedly in number in untreated abalone post June 1999 after the second spring/summer mud worm reproductive period. Treatment efficacy data for the two drying periods are reported in Chapter 5.1 (Trials 6 and 10).

In the second August 1998 intake cohort at Huon Aquaculture, low level shell blistering was also detected a month after transfer to the site. Blisters categorised as "active" dominated during the first spring until autumn the following year (1999). Mean total blister coverage reached approximately 35% by January 2000 and remained at this level until final sampling in November 2000 (Table 7.2).

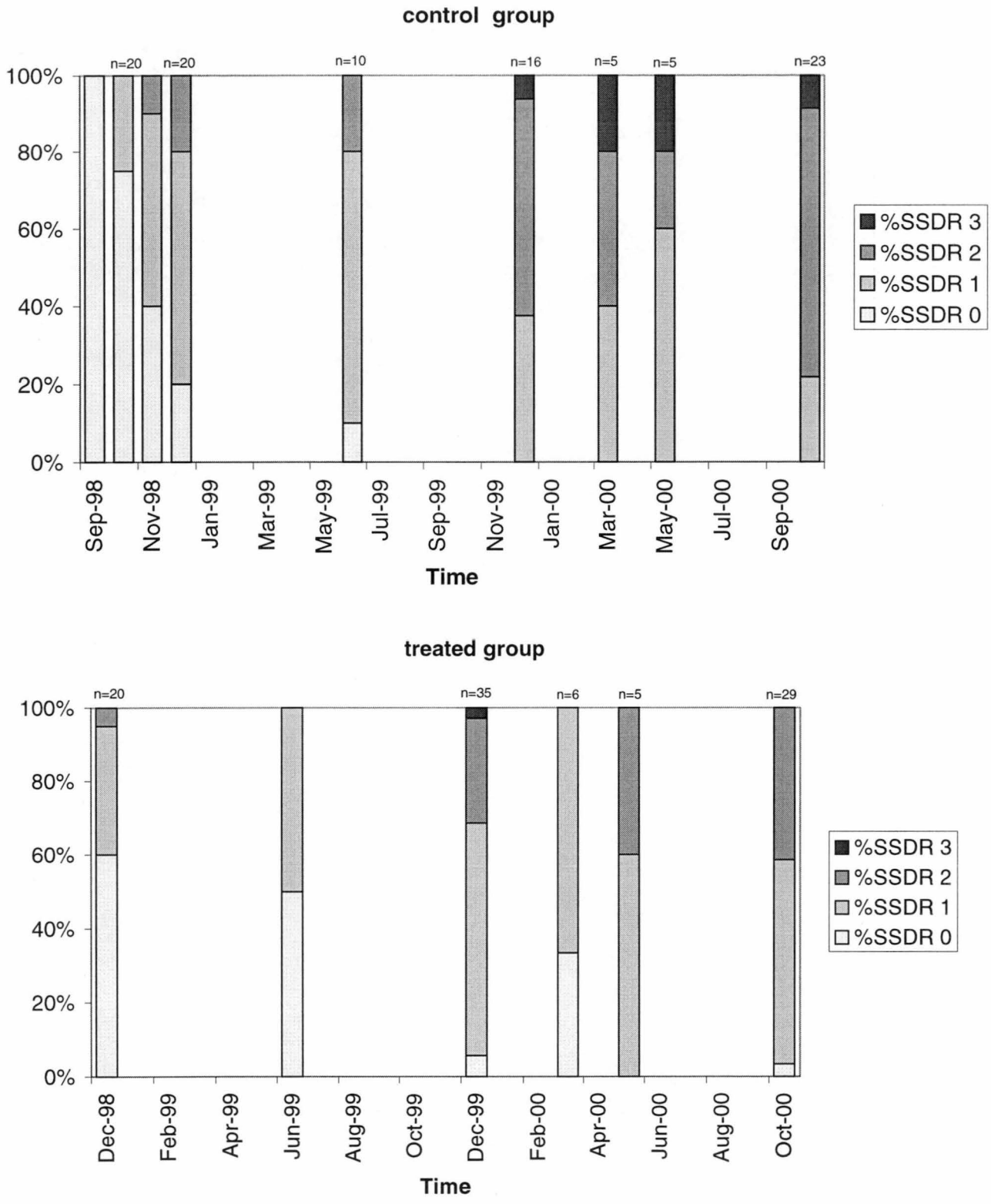
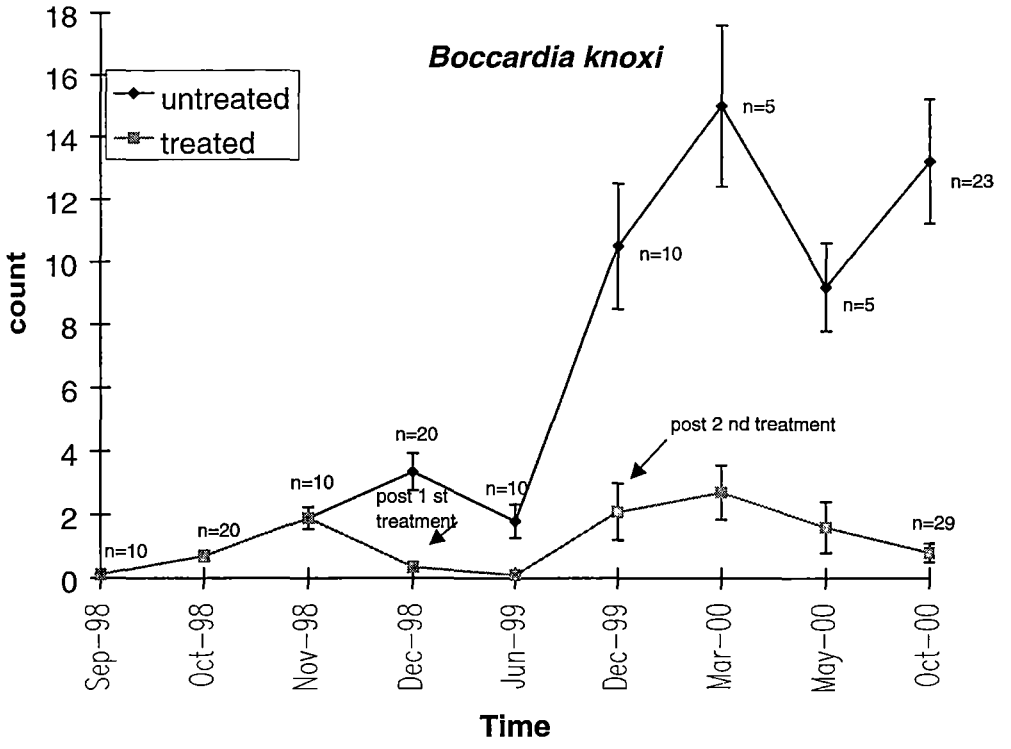


Figure 7. 14 Temporal change in Subjective Shell Damage Rating (SSDR) for Huon Aquaculture August 1998 Intake (Cohort 1).



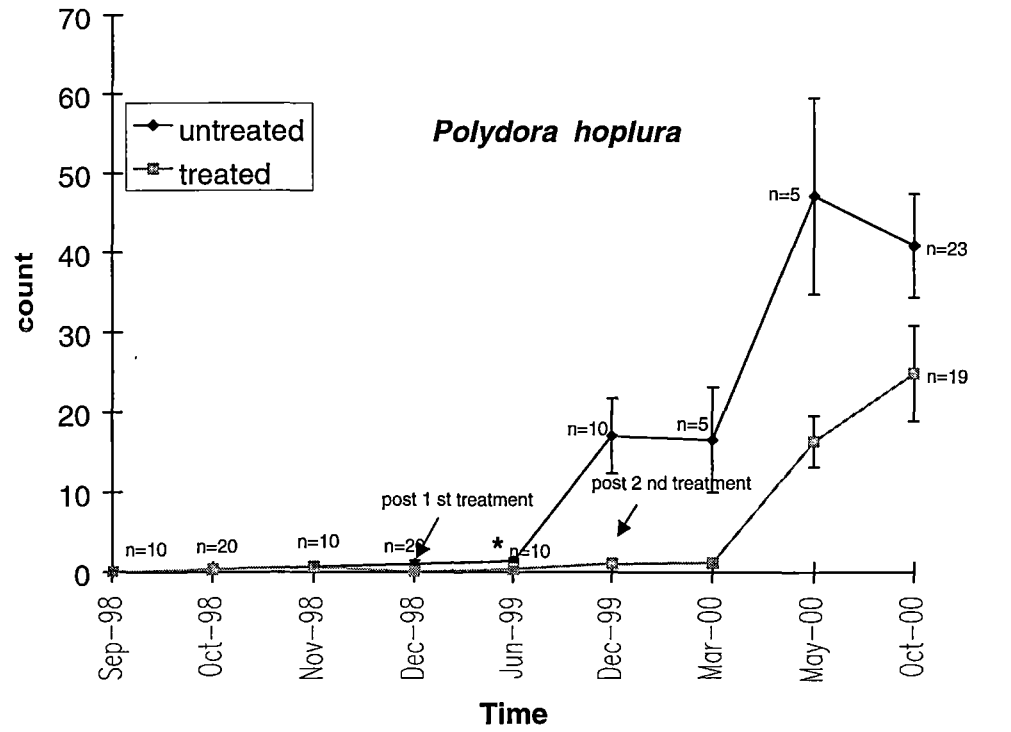


Figure 7.15 Mud worm counts for Huon Aquaculture August 1998 intake (cohort 1) treated and untreated abalone (means ± SE, n values equivalent for each data pair unless indicated Sample time data pairs marked with an * are not significantly different (P>0.05).

A significant inverse relationship existed between percent total blister cover and both final length (Figure 7.16) and whole weight of abalone (df 1 47, P<0.001 for both). Full regression analysis summaries including estimates of parameters and significance of r-values are shown in Appendices 7D and 7E.

The pattern of spionid infestation in cohort 2 at Huon Aquaculture was similar to that of the August 1998 cohort 1 untreated control stock until early 2000. By November 2000, however, total spionid numbers had increased in cohort two from approximately 50 to 160 worms per abalone (Table 7.2). Approximately one third of the *P. hoplura* were juveniles no more than 5 mm long and considered less than 3 months in age. Count data showed that *P. hoplura* was more common than *B. knoxi* in both August 1998 cohorts at this site, but more so in relative and absolute terms in cohort 2 as compared to cohort 1 (Table 7.2). This was attributed to an interaction between spionid reproductive strategy and rearing container type (Chapter 6.3).

At the Aquatas site, blistering of the August 1998 intake abalone increased steadily with little nacre present on blisters in the first year. Maximum blister coverage exceeded 25% in March and April 2000 as stock mortality was recorded (Figure 7.17). Maximum recorded blister levels were not significantly different (U=116, P>0.05, Mann-Whitney U-test) between similarly reared August 1998 intake cohorts at Aquatas (30.3% ± 9.0, 14) and Huon Aquaculture (28.7% ± 2.4, 23), ($\overline{X}$ ± SE, n). Final samples from Aquatas had a low proportion of healed blisters (<50%, Figure 7.17) compared to the comparable Huon Aquaculture samples (Figure 7.12). The subjective shell damage rating system indicated a rapid decline in the proportion of zero-rated (blister free) shells in the first 6 months after transfer. Rating 2 (moderately blistered) shells dominated by the late spring 1999 and a small proportion of severe blister ratings (SSDR = 3) occurred in shell samples after November 1999.

Spionid counts were significantly lower (U=0.0, P<0.01 Mann-Whitney U-test) for the last Aquatas sample in June 2000 (6.5 ± 1.3, n=10) as compared to a similar (May 2000) Huon Aquaculture sample (56.4 ± 8.6, n=5), ($\overline{X}$ ± SE). Spionid counts were generally <10 per sample for this intake at Aquatas (Figure 7.18).

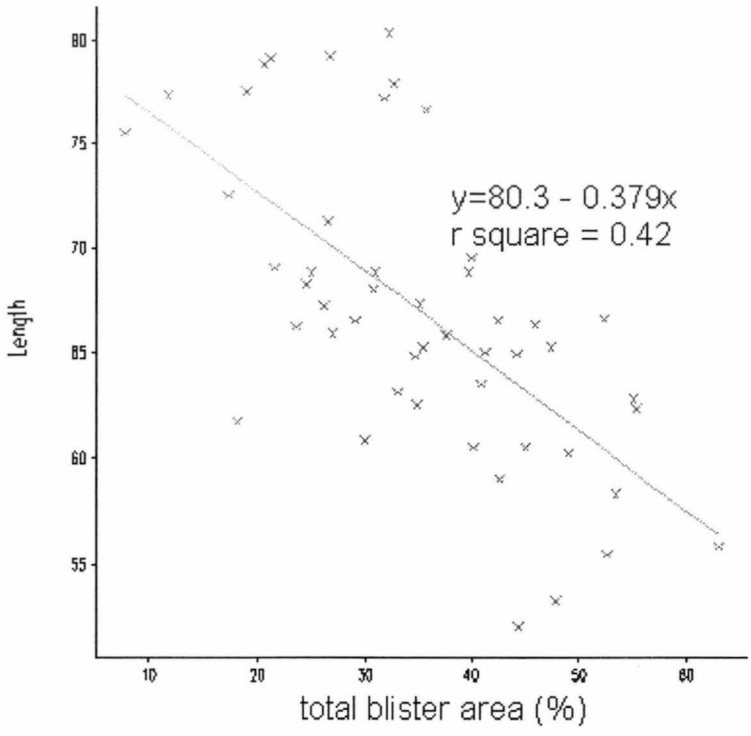


Figure 7.16 Total blister coverage versus length for Huon Aquaculture August 1998 (intake 2) abalone

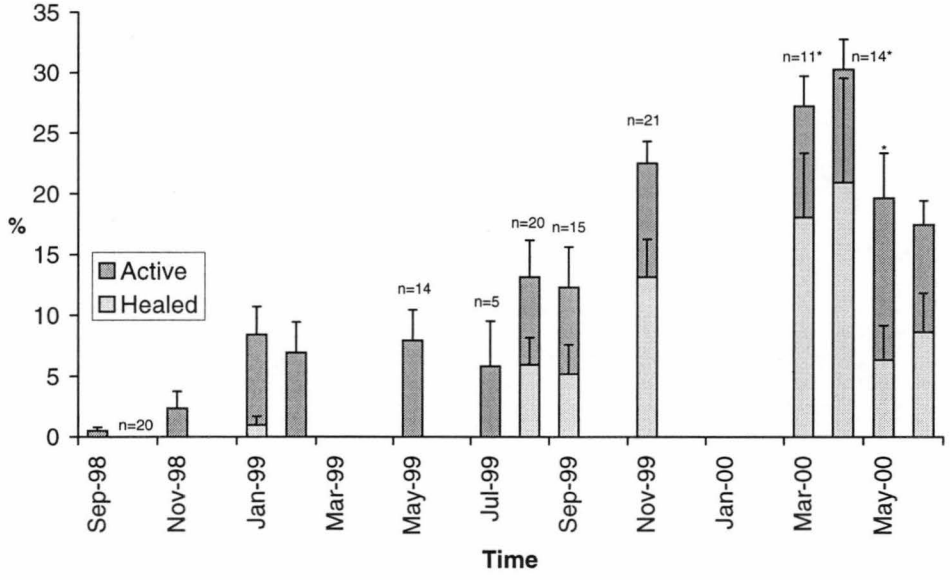


Figure 7.17 Progression and nature of shell blistering Aquatas August 1998 intake. Means ± SE, n=10 unless otherwise specified. * = sample of "dead" shells

Table 7.2 Final mean % blister coverage and mean spionid count per abalone at three farm sites, mean ± SE (n)

	Huon Aquaculture Company					
cohort	Aug 1998 (1)	Aug 1998 (2)	Spring 1998	Spring 1999	Dec 1999	Apr 2000
Date in	11/8/98	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	10/10/00	21/11/00	9/1/01	11/11/00	9/1/01	9/1/01
Time (d)	791	833	843	473	392	274
Shell blistering	28.7 ± 2.9 (23)	35.2 ± 1.8 (49)	1.9 ± 0.5 (64)	2.8 ± 1.5 (10)	0.2 ± 0.2 (10)	0.3 ± 0.3 (10)
B. knoxi	13.2 ± 2.0 (23)	2.1 ± 1.0 (49)	0.4 ± 0.1 (64)	0.1 ± 0.1 (10)	0 ± 0 (10)	0 ± 0 (10)
P. hoplura	40.9 ± 6.5 (23)	163.5 ± 10.0 (49)	0.8 ± 0.2 (64)	5.2* ± 1.7 (10)	0.1 ± 0.1 (10)	0 ± 0 (10)
Total spionids	54.1 ± 5.9 (23)	165.6 ± 8.3 (49)	1.2 ± 0.2 (64)	5.3 ± 1.7 (10)	0.1 ± 0.1 (10)	0 ± 0 (10)

Huon Aquaculture August 1998 intakes: (1) = control group from long term mud worm treatment experiment, (2) = stock reared in "tube" type containers
* most worms newly settled < 3 mm

	Aquatas				Tasmanian Scallops
cohort	Aug 1998	Spring 1998	Spring 1999	Dec 1999	April 2000
Date in	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	13/6/00	11/11/00	10/10/00	11/4/00	29/11/00
Time (d)	671	793	382	119	278
Shell blistering	17.5 ± 4.0 (10)	8.0 ± 2.0 (18)	3.1 ± 1.4 (10)	0.1 ± 0.1 (20)	0 ± 0 (10)
B. knoxi	3.1 ± 1.0 (10)	0.7 ± 0.2 (19)	2.3 ± 0.7 (10)	0 ± 0 (10)	0 ± 0 (10)
P. hoplura	3.4 ± 0.8 (10)	3.7 ± 0.7 (19)	0.1 ± 0.1 (10)	0 ± 0 (10)	0 ± 0 (10)
Total spionids	6.5 ± 1.3 (10)	4.4 ± 0.6 (19)	2.4 ± 0.7 (10)	0.1 ± 0.1 (10)	0 ± 0 (10)

Although, as previously indicated maximum blister levels were similar to more infested stock at Huon Aquaculture.

Other intake time data

Spionid infestation and subsequent shell blistering was minimal in stocks transferred to Huon Aquaculture and Aquatas in the spring months of 1998, 1999 and December 1999 (Table 7.2). After exposure to 3 successive spring/summer spionid dispersal periods, the Huon Aquaculture spring 1998 intake had <2 spionids per abalone by January 2001 (Table 7.2); significantly less infestation (U=0.0, P<0.01 Mann Whitney U-test) than the lesser infested of the two August 1998 intakes at Huon Aquaculture (Figure 7.15, Table 7.2). Mean blister coverage was similarly low at <2% by January 2001. This spring 1998 intake group grew from 18 to 58 mm during the study period (Table 7.1) but spionid infestation did not increase significantly with size. Placement of abalone at study sites in December 1999 to avoid presumptive *B. knoxi* settlement (Chapter 3) was successful at Huon Aquaculture with no worm of this species recorded by January 2001. Similarly no *B. knoxi* was recorded at Aquatas up to and including April 2000, following which time the baskets were lost (Table 7.2).

Stock present at Tasmanian Scallops on the east coast of Tasmania were sampled 7 times (n=10 per sample) between April 2000 and January 2001. Both *B. knoxi* and *P. hoplura* were present in the cumulative total of 4 adult spionids and 10 recently settled larvae. Only 1 mud worm blister, covering 10% of the shell was detected.

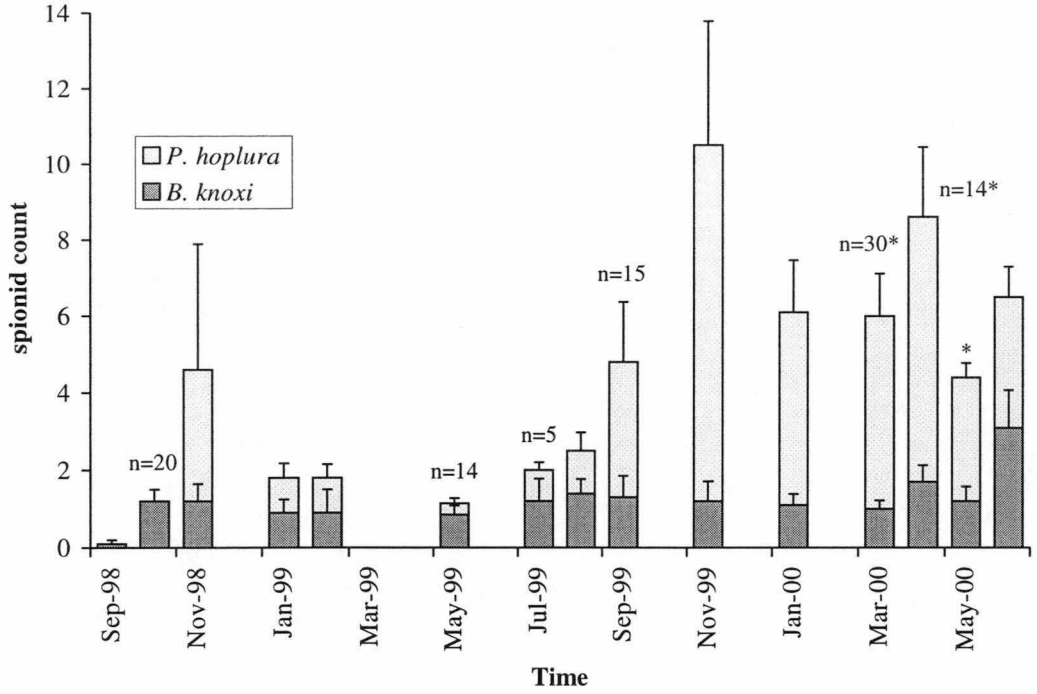


Figure 7.18 Temporal change in spionid count and species mix in August 1998 abalone intake at Aquatas. Means + SE, n=10 unless otherwise shown.

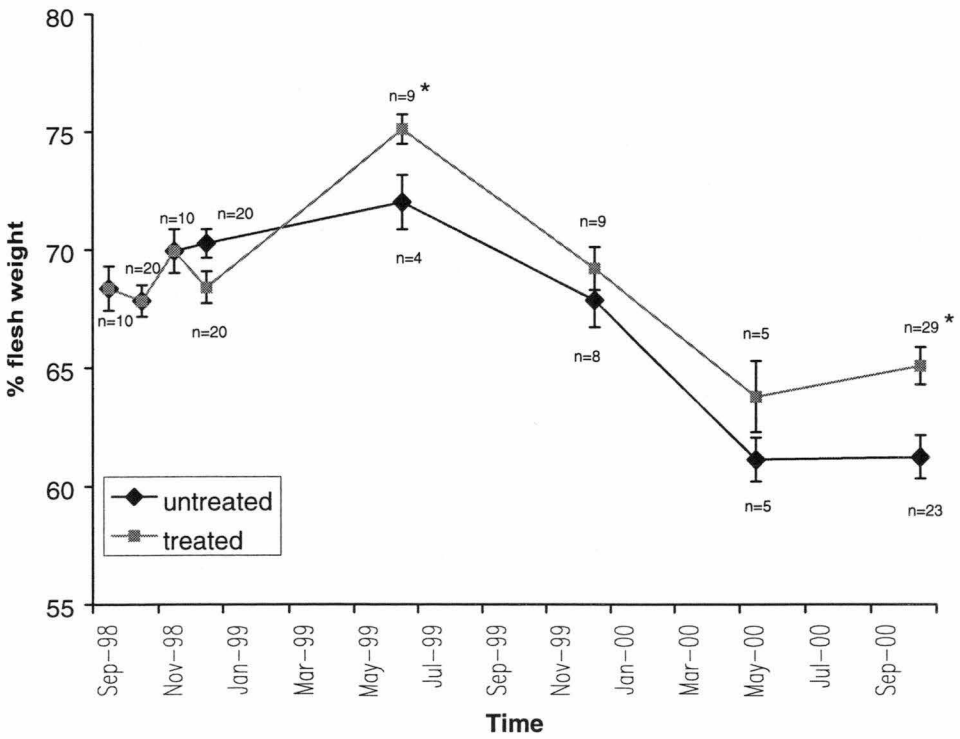


Figure 7.19 Percentage flesh weight comparison: Treated and untreated abalone, August 1998 intakes (cohort 1) at Huon Aquaculture (means ± SE, n values shown.) Data pairs denoted * are significantly different (P<0.05).

7.3.5 Dry weight indices

Preliminary assessment of various condition indices was made to ascertain the most suitable for long-term health monitoring. Regression analysis showed there was a highly significant relationship between weight and CI_{LENGTH} (df 1 41, $P<0.001$, Appendix 7F) with the condition index increasing with weight of stock. Further, the dry weight index CI_{LENGTH} increased as a function of shell length (df 1 41, $P<0.001$, Appendix 7G). Thus, as CI_{LENGTH} increased with stock size it was unsuitable for long term-term studies where growth was expected.

By contrast, there was no significant relationship between length and percentage wet flesh weight (df 1 41, P=0.401, Appendix 7H) and weight and percentage flesh weight (df 1 41 P=0.361, Appendix 7I). In both regressions the residual variance exceeded the variance of the response variance (percentage flesh weight) and no r-squared value could be calculated.

Regression analysis of shell length and CI_{WEIGHT} was not statistically significant (df 1 41, P=0.073, Appendix 7J) and nor was that between whole wet weight and CI_{WEIGHT} (df 1 41, p=0.107, Appendix K). Regression analysis of percentage dry flesh weight data and length was statistically significant (df 1 41, P=0.006, Appendix 7L) as was that between dry flesh weight data and weight (df 1 41 P=0.039, Appendix 7M). Thus, this measure of abalone condition was confounded by differences in size of animals and of little use.

Although the probability values for the likely hood of a significant relationship between CI_{WEIGHT} and the two measures of abalone size were >0.05, they were much closer to being statistically significant than those of percentage flesh weight and abalone size, measured by weight and length. Thus, percentage flesh weight was felt to provide a better measure of relative "fleshyness" or condition than either dry weight condition index.

7.3.6 Percentage Flesh Weight

A normal range for percentage flesh weight data was established by calculating means for 24 samples of 10 abalone between 20 and 70 mm in length. Mean percentage flesh weight was 71.1% (SD=2.1%, n=24). By contrast, an assessment of percent flesh weight on remnant stock infested with mud worm in the mid-1990's and held at Tasmanian Tiger Abalone showed mean percent flesh weight of 52.6 (SD=3.1%, n=10). These abalone had blistering to approximately 25% of their shells with approximately one third having the maximum SSDR of 3. Data for abalone transferred and spionid infested during 1998-2001 is presented below.

Huon Aquaculture August 1998 cohort 1

Two-way ANOVA after arcsine transformation of percent flesh weight data showed P values of <0.001 and 0.007 for time and treatment as factors, respectively (ANOVA Table, Appendix 7N). Air-dried stock samples were approximately three percentage points higher than those of non-treated control stocks following the initial December 1998 treatment episode (Figure 7.19) were. This differential was maintained throughout the length of the study. Percentage flesh weight of treated and untreated stock fell from peak values in May 1999 (Figure 7.19). This coincided with decrease in growth and increases in blistering and mud worm counts for both air-dried and untreated stock. The final tissue weight of the untreated control stock was 61.2% (SD=4.3, n=23), significantly lower (P<0.01, U=18.0, Mann-Whitney U Test) than the mean of 71.1% (SD=2.1%, n=24) established for the apparently healthy abalone.

Percentage weight data for other stock cohorts

Huon Aquaculture abalone percent flesh weight final values for the various cohorts were generally 68% or higher. One exception to this trend was the untreated control group (61.2% as described above) which contrasts with the other August 1998 intake (2) from the site with a value of 67.5% flesh weight (Table 7.3). Thus, this latter group of abalone maintained a percentage flesh weight value close to the normal range despite having mean blister coverage of 35% and mean spionid counts of greater than 150 (Table 7.2). The August and spring 1998 intakes at Aquatas had final percentage flesh weight figures of 51.3 and 46.4%, respectively (Table 7.3).

Table 7.3 Percentage flesh weight data for abalone at three farms

	Huon Aquaculture Company					
Cohort	Aug 1998 (1)	Aug 1998 (2)	Spring 1998	Spring 1999	Dec 1999	Apr 2000
First sample	Sep 1998	Sep 1998	Oct 1998	Oct 1999	Feb 2000	May 2000
Last sample	Oct 2000	Nov2000	Jan 2001	Nov2000	Jan 2001	Jan 2001
Initial % flesh weight $\overline{X}$ ± SD (n)	68.4 ± 3.0 (10)	68.4 ± 3.0 (10)	65.1 ± 6.2 (20)	70.6 ± 1.7 (10)	68.1 ± 4.8 (10)	71.4 ± 1.9 (10)
Final % flesh weight $\overline{X}$ ± SD (n)	61.2 ± 4.3 (23)	67.5 ± 4.3 (49)	71.2 ± 4.8 (54)	66.8 ± 4.2 (10)	69.2 ± 2.4 (10)	73.6 ± 1.6 (10)
Final % blister coverage	31	35	2	3	<1	<1

	Aquatas				Tas. Scallops
Cohort	Aug 1998	Spring 1998	Spring 1999	Dec 1999	April 2000
First sample	Sep 1998	Oct 1998	Oct 1999	Jan 2000	Jun 2000
Last sample	Jun 2000	Nov 2000	Oct 2000	Apr 2000	Nov 2000
Initial % flesh weight $\overline{X}$ ± SD (n)	71.8 ± 1.8 (10)	66.2 ± 5.0 (20)	71.8 ± 2.9 (20)	63.9 ± 2.9 (20)	74.1 ± 2.8 (10)
Final % flesh weight $\overline{X}$ ± SD (n)	51.3 ± 17.4 (10)	46.4 ± 9.5 (10)	64.3 ± 3.7 (10)	64.4 ± 4.4 (20)	73.7 ± 2.3 (10)
Final % blister coverage	17*	8	3	<1	0

* = final sample value, peak value was 30%

7.4 Discussion

Early pilot scale, sea-based abalone grow-out ventures in southern Tasmania were severely compromised by stock mortality associated with high spionid mud worm counts and shell blistering. With one exception, in the present study, there was little evidence of mud worm related mortality in stock transferred to two sites with a previous history of severe infestation. Some stock groups were exposed to mud worms for up to 27 months, had mean infestation levels in excess of 50-150 worms per animal, and blistering to approximately one third of the shell area, yet did not suffer the mortality episodes previously seen at the site.

Stock mortality occurred in one intake time group (August 1998) at only one site. Shell blister damage levels were similar in this intake time group at both sites but there was steady, albeit, slow growth of the stock at one site but not the other. Mortality at the latter site began approximately 18 months after initial stocking and subsequent spionid colonisation. Growth data indicated suppressed growth at the Aquatas site generally and this was considered to be largely due to relatively infrequent feeding. However, relatively poor nutrition alone, in the absence of significant spionid infestation did not result in mass stock mortality. Thus, it appeared that high levels of shell blistering in addition to poor nutrition resulted in stock mortality; whereas, high levels of spionids and shell damage in the presence of adequate nutrition did not result in significant mortality at Huon Aquaculture. Possible reasons for the mortality of uninfested stock soon after transfer to Huon Aquaculture in spring 1999 and 2000 were the greater travelling time to reach this site and/or the presence of predatory fish outside the rearing containers at Huon Aquaculture but not Aquatas.

The effect of spionid infestation on growth rate of abalone has not been addressed in previous studies on wild stocks but is of considerable interest to the grower. The comparison between spionid treated (air-dried) and infested control abalone showed that the air-dried stock grew significantly faster in the 7 months post treatment (Dec 1998-Jul 1999-section 7.3.2). The suppression of the growth

rate in the untreated infested stock was estimated at 20%. Maximum spionid infestation associated with this growth depression consisted of four worms per abalone corresponding to blister coverage of 7%. Growth depression was also seen in Chapter 6.2 where spionid infestation with 3-5 worms and subsequent blistering in spirorbid-fouled stock caused a mean 28% reduction in shell growth compared to minimally infested control stock. Regression analysis on heavily infested abalone (Huon Aquaculture, August 1998 intake cohort 2) showed that size of abalone defined as both length and weight declined with increased blister cover.

Of the many stock cohorts placed at the study sites for temporal health studies (reported in this chapter), risk factor analysis (Chapter 6) or larval dispersal experiments (Chapter 3), only the August 1998 intakes became heavily blistered. Blister coverage of approximately 30% was seen in the present study as in the previous abalone mortality episodes that led to this research. However, while blister coverage levels were similar, SSDR data from remnant stock surviving the original mortality episode until 1997-1998 (Appendix 1) suggested the original shell damage episodes were more severe. Previously, blisters were often very soft and consisted of 5 mm or more of apparent conchiolin material over the ventral surface of the shell. Blisters of this nature were absent during the course of the present study and the maximum SSDR score was rarely used. Large blisters in the present study were usually covered with a hard layer of pearly nacre. Possibly factors such as nutrition could account for some of the differences between blisters in past and present episodes. Certainly, in Australia considerable research and development effort has gone into the area of formulated abalone diets since the original spionid outbreaks.

Blisters formed by *B. knoxi* worms were frequently located in the shell apex. This was consistent with the finding (Chapter 6.2) that chimney structures created by this species were often located in the groove under the shell apex. It would, therefore, appear that *B. knoxi* is capable of burrowing directly through the shell of an abalone. Blake and Evans (1972), in reviewing mode of spionid blistering, noted that two routes of host invasion were reported. In the first the larvae swim into the mantle cavity or burrow between the shell edge and the mantle. In the second

spionid larvae settle on the outside and penetrate directly. This latter method is undoubtedly favoured by *B. knoxi* with initial settlement of the apical groove consistent with the report of Zottoli and Carriker (1974) that *P. websteri* larvae settle wherever there are crevices in oyster shells. The respiratory pores of abalone appear to be another favoured site for spionid settlement. Whether this is solely because they present a shell irregularity and, thus, a degree of protection or because spionid larvae actually enter the mantle cavity by this means is unclear. There is no doubt that *P. hoplura* larvae can enter the abalone shell through the leading edge but the diversity of blister locations suggests they may also be capable of burrowing directly through the shell. Some second generation larvae appear to never leave the shell (Chapter 3) creating new burrows adjacent to the maternal blister.

As noted earlier, the majority of the different intake time cohorts suffered negligible spionid infestation. The spring 1998 intake at Huon Aquaculture, stocked at 18 mm was exposed to three full or partial spring/summer mud worm settlement seasons and acquired an average of only 1 spionid per abalone. Interestingly, this group was minimally infested after the first spring exposure and remained so despite subsequent growth to 58 mm (Table 7.1). The only stocks that became heavily spionid infested were the August 1998 intakes at both study sites. These animals have since been shown to be at higher risk due to their initial fouling with spirorbids (section 2.1, this chapter), shell irregularities and greater stocking size.

Five mud worm species were found to infest stock in the present study: *B. chilensis*, *B. knoxi*, *B. proboscidea*, *P. armata* and *P. hoplura*. Of these, only *B. knoxi* and *P. hoplura* were present in sufficient numbers to be considered pest species. Interestingly, *P. hoplura* was far more numerous than *B. knoxi* in this study. This is the reverse of the pattern seen in the remnant stock from Huon Aquaculture that was used in the treatment options studies (Chapters 4 and 5). Of the three minor species *P. armata* has been previously recorded in *H. rubra* from Victoria and in *H. roei* from South Australia (Blake and Kudenov 1978). *Boccardia chilensis* is quite common in Tasmanian oysters, but neither *B. proboscidea* nor *P. armata* were previously known from this source (Wilson et al. 1993).

A review of spionids found in abalones worldwide is included in Appendix 7O. A major previous study was conducted by Kojima and Imajima (1982) who found four species of spionids in *H. diversicolor* from Japan (Appendix 7O). Of these *P. websteri* had the highest frequency of occurrence in stock but *P. armata* was the most abundant worm over all. The *P. websteri* species is the most common species in Tasmanian oysters (Wilson et al. 1993). Few of the 20-70 mm wild abalone studied by Kojima and Imajima (1982) had greater than 20 spionids per abalone, substantially less than the extremes of infestation seen in the Huon Aquaculture August 1998 intakes. As in the present study Kojima and Imajima (1982) found some specialisation among worm species in the part of the host shell settled.

Horne (1996) found between 50 to greater than 100 spionids per abalone in *H. kamtschatkana* from British Columbia. These abalone had mean length of 106 mm and were infested with a species tentatively assigned to *P. ligni*. This is comparable to a survey by Sinclair (1963) of wild *H. iris* in New Zealand who found up to 100 *P. monilaris* (considered to be equivalent to *P. armata* by Read 1975) in larger abalone. Infestation at this level was reported to ruin the shell for commercial purposes. Clavier (1989), in a study of wild *H. tuberculata* from the Brittany coast of France, found abalone greater than 80 mm were infested with a mean of approximately 40 worms per shell of five spionid species (Appendix 7O). As in the present study *P. hoplura* was the most numerous accounting for 77% of all spionids.

Clavier (1989), by the use of a condition index found no physiological effect of mud worm infestation on abalone. However, the author suggested that extreme infestations (not considered in the random sampling method used) were certainly detrimental to *H. tuberculata*. Kojima and Imajima (1982) found that 10 spionids per abalone significantly reduced the flesh weight of affected individuals. In the present study percentage flesh weight declined significantly with time (as blistering increased) and in untreated infested stock compared to less infested, air-dried stock. The severe declines in percentage flesh weight seen previously (to 53% in remnant stock from original mortality reports) were not observed in this study, with the

exception of some cohorts that were underfed rather than heavily infested. These latter included the August 1998 and spring 1998 intakes at Aquatas. Considerable variability exists in the literature regarding lethal and sublethal effects of spionid mud worms in molluscs. Whitelegge (1890) investigated mass mortality in Sydney rock oysters (*Saccostrea commercialis* Iredale and Roughley), associated with *P. ciliata*, but later classified as *P. websteri* (Blake and Evans, 1972). Whitelegge recommended changes in culture techniques including lifting oysters off the substrate and air exposure at low tide. These methods were highly effective and are still used today. Smith (1982, 1984) reviews the collapse of the New South Wales and Queensland oyster industries in the late 1880's and early 1900's and attributes a great part of the blame to mortality caused by spionid mud worms. Korringa (1952) reviewed spionid infestation outbreaks in oysters from the 1940's and early 1950's, finding only one instance of widespread mortality.

Korringa (1952) states that when oysters contained over 25 *P. ciliata* or over five *P. hoplura* they grew poorly and were often leaner than non-infested oysters. The *P. hoplura* species is among the largest of the spionids that infest shellfish and is usually considerably larger than *B. knoxi*. Therefore, if larger worms cause greater damage to the host, then infestation by ~35 *B. knoxi* (as seen in remnant stock from the original outbreak examined in 1997- Chapter 1) should be less serious than infestation by 150 *P. hoplura* (as seen in this study). Yet as this was not apparently the case it may be suspected other factors are involved.

The work of Owen (1957) contrasts with reports attributing oyster mortality to high levels of mud worm infestation. In this study 100% of the oysters studied were infested and *P. websteri* numbers exceeded 100 per host at some study sites. The author concluded that although in some areas a positive correlation was found between oyster mortality and degree of infestation this was not confirmed in controlled laboratory experiments. He concluded that *P. websteri* does not cause oyster mortality per se but contributes towards the formation of a poor environment.

Sub-lethal impacts of spionids on bivalve molluscs have been reported by several authors. Kent (1979) found that heavy infestations of *P. ciliata* were

associated with reduced flesh content in mussels. Wargo and Ford (1993) found a significant negative correlation between percent blister coverage and condition index in the oyster species *Crassostrea virginica*. When half of the shell was blistered the condition index of oysters was reduced by up to one third. This study concluded that spionid infestation reduced the ability of the host to accumulate nutritional reserves. Handley (1997) found that *B. knoxi* produced a statistically significant negative impact on condition of sub-tidally cultured oysters but that the effect was too weak to have any biological significance. Cacerez-Marinez et al. (1999) found that heavy infestation of the black clam *Chione fluctifraga* Showerby with *Polydora* sp. could weaken the shell leading to increased predation.

In an earlier study Caceres-Martinez et al. (1998) found no correlation between *Polydora* sp. numbers and blister area with condition of the oyster species *C. gigas*. However, infestation levels were light with generally 1-3 worms recorded per shell. Similarly, low level infestations (generally < 5 worms per shell) in intertidally cultured *C. gigas* did not have any significant biological impact as assessed by Handley and Bergquist (1997). Low level infestation by *P. ciliata* in the Indian oyster *Crassostrea madrasensis* Preston was not reported to seriously injure the host (Stephen, 1978). Heavy infestations of a mixture of spionids were reported in the Japanese scallop *Patinopecten yessoensis* by Sato-Okoshi and Nomura (1990) but few health effects if any were not mentioned. Contrasting conclusions in these previous studies as to the health impacts of spionids on molluscs may be due, in large part, to differences in the severity of infestation (measured by spionid count or blister damage), the size of the host, host species and other environmental factors such as food availability.

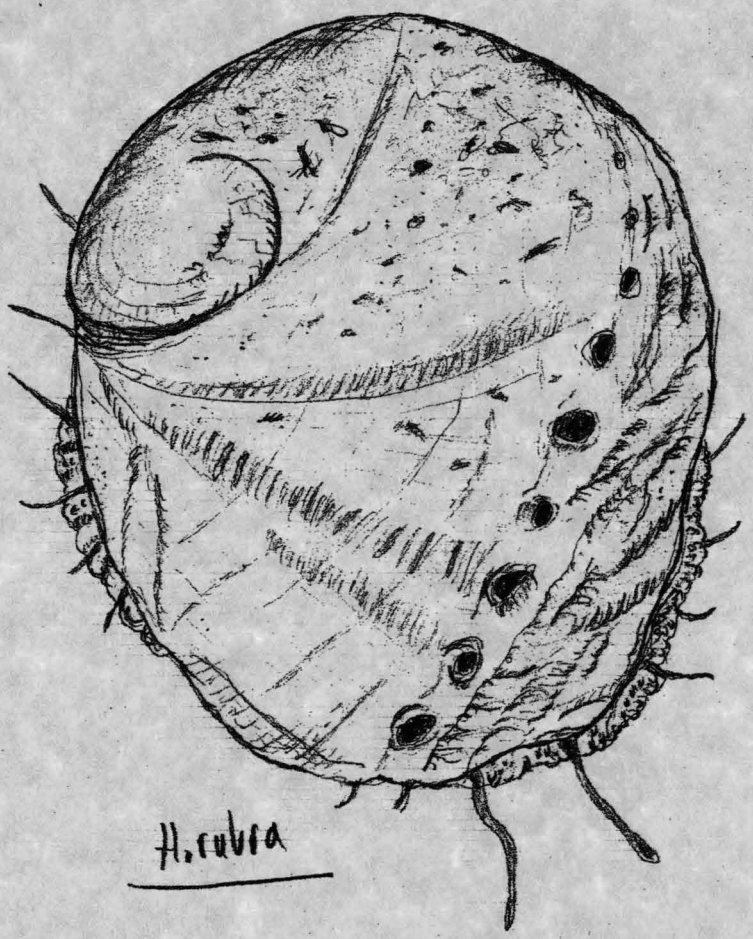
The long-term treatment trial involving air drying in December 1998 and 1999 was highly effective in reducing infestation by *B. knoxi*. Unfortunately *P. hoplura* rather than *B. knoxi* was the more numerous polychaete in this study - reversing the pattern seen during the original mortality episodes. Some post-treatment infestation by *P. hoplura* meant that stock did not remain essentially mud worm free between treatments. Even so, the treatment regime resulted in lower infestations with

P. hoplura, less blister damage and higher percentage flesh weight in treated as opposed to untreated control stock. The treated, air-dried stock also grew faster in the initial 7 months post treatment.

As relatively moderate spionid infestation was shown to suppress growth, monitoring of stock to ascertain mud worm levels is prudent in future farming situations. The decision to treat or not would be based on level of spionid infestation, growth rate of stock and final market size required. Stock placed in December 1999 at both study sites recorded little spionid infestation and none by *B. knoxi*. Use of this strategy should confer almost a year's growth free from the affects of spionid infestation. This may be sufficient so that any subsequent infestation would have minimal impact before stocks were sold negating the need for treatment.

7.5 Conclusion

The majority of abalone intake time cohorts transferred to the two southern Tasmanian study sites failed to become significantly mud worm infested. Despite that, some abalone were exposed to up to three successive annual spring/summer spionid larval dispersal periods. This is a positive finding for industry indicating that spionid infestation of stock may be a comparatively rare event. Where abalone stocks did become heavily infested they appeared, in retrospective, to have been at increased risk of infestation in terms of size and shell fouling (Chapter 5). None the less these stocks were able to sustain very high mud worm numbers and associated shell blister coverage for up to two years without significant mortality. Where stock mortality did occur abalone were exposed to poor husbandry conditions in addition to high levels of shell blistering. Spionid infestation was shown to potentially cause growth suppression and reduced flesh yield. A post-larval settlement air-drying treatment regime reduced spionid numbers and associated blistering. Monitoring of mud worm levels in future farmed stock from susceptible areas is prudent so decisions can be made as to whether treatment is appropriate.


H. rubra

Frontispiece: live blacklip abalone

Chapter 8
CLINICAL PATHOLOGY, HISTOLOGY AND OTHER INDICATORS OF ABALONE HEALTH STATUS

8.1 Introduction

As noted in the preceding chapter there is a paucity of literature on spionid mud worm infestations in abalone, particularly concerning health affects other than growth or condition indices as were reported in Chapter 7. Parasites can cause alterations from the normal state as evidenced by histopathological, physiological and biochemical changes (Malek and Cheng, 1974). This chapter reports on such changes as a result of abalone mud worm infestation for field studies in the period 1998-2001 and for remnant stock from the original reported mortality episodes (Chapter 1).

Alterations in haemolymph electrolytes in abalone were reported by Harris (1999) in relation to water quality stresses. Gastropod molluscs, such as abalone, are generally considered to be osmoconformers but there is growing evidence that at least some ions are regulated (Burton, 1983). Consequently, these may have potential as health indicators and the effects of mud worm infestation on clinical pathology indicators such as haemolymph electrolytes, copper, protein and glucose were examined. Copper-containing haemocyanin is the main respiratory pigment in gastropods (Bonaventura and Bonaventura, 1983) including abalone (Ainslie, 1980). Thus, measurement of haemolymph copper gives an indirect measure of respiratory pigment level and has previously been assessed in abalone under stress by Harris (1999). Haemolymph variations in protein and glucose in response to parasitism in molluscs have been reported by Malek and Cheng (1974) and, consequently, are measured here comparisons made with spionid infested animals. This chapter also describes histology of abalone surviving from the time of the original reported outbreaks and documents changes in tissues of abalone experimentally exposed to mud worm infestation. Variations in tissue composition have previously been

reported in diseased abalone (Kismohandaka et al., 1995) and, consequently, tissue glycogen and protein levels were assessed in the present study by histological methods and/or direct measurement. Aspects of physiology such as respiration rate have been used as an indicator of metabolic stress in molluscs (Edwards, 1996) including exposure to parasites (Malek and Cheng, 1974) and consequently is used in the present study. Likewise, ammonia excretion has been seen to alter in diseased abalone (Kismohandaka et al., 1993) and is reported here. The microenvironment of the blister regarding potential health effects from blister fluid pH and the presence of harmful microflora such as bacteria and fungi was also examined. Changes to "normal" abalone structure and function as a result of mud worm infestation may have wider potential as general indicators of abalone health.

8.2 Materials and Methods

8.2.1 Experimental animals and holding conditions

Unless otherwise specified experimental abalone were originally acquired from farm 1 and transferred to sea based farms belonging to either Aquatas or Huon Aquaculture and located in the south of the state (Chapter 2). Holding conditions are previously described in Chapters 2 and 7. Abalone experimentally starved for 3 months were held at the Animal Health Laboratory, Launceston (Chapter 2) and sampled every four weeks (section 8.2.8).

8.2.2 Clinical Pathology

Levels of sodium, potassium, magnesium, calcium, chloride, glucose and protein in haemolymph were determined using a Cobas-Mira auto-analyser (Chapter 2). Haemolymph copper was assessed using atomic absorption methods and samples were taken from either the cephalic sinus or foot as indicated (Chapter 2). Background data on normal levels for non-spionid infested abalone was established by sampling groups of 5-10 presumptive healthy animals acquired in the period

1998-2000. Animals were growing well upon collection and had no visible health problems. The abalone were obtained from farm 1 or Tasmanian Scallops on the east coast (Chapter 2). These apparently healthy abalone were compared to mud worm infested stocks from Huon Aquaculture, Aquatas and Tasmanian Tiger Abalone. Animals assigned to the mud worm affected group were collected 1998-2000 and comprised moderately to severely infested animals. The August 1998 intakes of abalone to Huon Aquaculture and Aquatas were sampled at regular intervals until mid- late 2000. These groups were subsequently shown to be the most severely mud worm infested (Chapter 7) and, thus, of most interest for clinical pathology investigations.

Statistical methods for comparison of data varied with the size and nature of data sets. The non-parametric Mann-Whitney U Test was used to compare serum electrolytes and copper, protein and glucose in haemolymph withdrawn from the cephalic sinus of presumptive normal and mud worm infested stock. As a larger data set was available for haemolymph withdrawn from the foot, distributions approached the normal distribution and the more powerful t-test was used. Similarly, the distribution-free, Kruskal-Wallis test was used to assess Na^+/K^+ ratios of the relatively small data set of starved animals (section 8.2.8), whereas a one way ANOVA was used to assess the larger temporal variation data sets from the two study sites. Long term, treatment trial data (August 1998 cohort 1, Huon Aquaculture- Chapter 7) were tested with two way ANOVA, with sample time and treatment as factors. Seawater samples from the abalone farm study sites were tested by Cobas-Mira auto-analyser to determine levels of sodium, potassium, magnesium, calcium, and chloride ion.

8.2.3 Histology

Routine histological processing with haematoxylin and eosin staining was performed as described in Chapter 2. Tissues routinely examined included right kidney, digestive gland, intestine, gills and foot. Some comparisons were made with Martius Scarlet Blue (MSB) (Ellis, 1992) stained sections to examine depletion of foot muscle in spionid infested abalone. Sections of uninfested and severely mud worm infested abalone were stained using the Periodic Acid Schiff reaction (PAS) (Ellis, 1992) to check for fungi and tissue glycogen levels.

Remnant, severely mud worm infested abalone stocked at southern sea farms in the mid-1990's were sampled for histological examination in the period 1998-99. These changes were compared to changes that occurred when mud worm free stock were transferred to the same areas in August 1998 and September-November 1998. A subjective score was developed to assess the extent of brown pigment granules present in the right kidney and digestive tubules of abalone sampled. A zero score indicated no pigment and a score of 3 indicated maximum pigment levels.

8.2.4 Blister environment and microflora

The pH of a sample of 10 large blisters was measured by withdrawing at least 0.2 ml of blister fluid with a syringe and needle and wetting the tip of pH test strip (range 4.5 -10.0, Sigma). Blisters tested came from remnant stock surviving the initial reported outbreaks in the mid-1990's until sampling in 1998-99.

Similarly, samples of blister fluid from some of the most mud worm damaged shells available were cultured for bacteria on TCBS, Johnson's Marine Agar and Blood Agar plates and incubated at 20 °C (AHL Fish Health Methods Manual, 2001). Abalone shells and adjacent tissue were processed for histology and stained using Periodic Acid Schiff reaction (PAS) (Ellis, 1992) to test for the presence of fungi. Shells were decalcified using RDO rapid bone decalcifier (Phoenix Scientific, Vic., Australia).

8.2.5 Tissue chemistry

Foot tissue was frozen and stored at –20°C and soluble protein content measured using a Sigma Diagnostics protein assay kit (Procedure No. P5656) based on the Lowry method. Sample preparation involved placement of 0.05-0.15 g frozen tissue in cold SEI buffer (250 mM sucrose, 5 mM EDTA in 0.1 M imidazole buffer). Tissue was then homogenised with a teflon rod while kept on ice. Homogenate was centrifuged for 7 min at 1370 g at room temperature and the supernatant discarded. The remaining pellets were resuspended in 1 ml SEID solution (consisting of SEI plus 0.1 g sodium deoxycholate per 100 ml SEI) re-homogenised, and left on ice for 15 min. The homogenate was then centrifuged for 6 minutes at 3000 g and a 0.2 ml sample of supernatant diluted to 1 ml as per protein test kit method. Absorbency was measured at 700 nm with a light spectrophotometer and the protein concentration determined from a standard curve. Data were calculated as mg protein per gram tissue as expressed at percent protein wet weight foot tissue. Statistical analysis of tissue protein data were performed using the non-parametric- Kruskal-Wallis test in conjunction with non-parametric Tukey-type multiple comparisons to separate means (Zar, 1984).

8.2.6 Respirometry

Respirometry trials were performed at University of Tasmania, Launceston in March 1999 and May 2000. In the first trial remnant mud worm infested stock from the mortality events of the mid-1990's were sourced from Huon Aquaculture and compared to mud worm-free stock of similar age from farm 1. Mean shell lengths were 57.8 mm (SD = 2.7, n=8) and 58.1 mm (SD=7.4, n=4) respectively. Abalone selected for the second experiment were part of the long-term treatment trial at Huon Aquaculture. Five mud worm infested animals with spionid counts of 62.8 ± 24.4 and blister coverage of 18.2% ± 9.8% ($\overline{X}$ ± SD) were compared to five less infested stock (previously air dried December 1998 and 1999) with spionid counts of 18.4 ± 5.8 and blister coverage 12.9% ± 9.0 ($\overline{X}$ ± SD).

Mean lengths of the untreated and less infested groups were 61.9 ± 3.6 mm and 64.9 ± 5.1 mm respectively.

After selection and transport abalone were held for 10 d and 7 d without food, respectively in trials one and two. Previous experience (Chapter 4) with the remnant heavily infested stock used in trial 1 had shown the potential for mortality following transport to the laboratory. Thus, a prolonged observation period was allowed post-transport for recovery and to avoid selection of moribund animals. Feeding was considered to potentially compound the stress in highly spionid infested, recently transported abalone, and was omitted. For consistency all other abalone, including uninfested and relatively uninfested controls, were treated likewise. Animals chosen for the respirometry studies had negligible shell fouling organisms.

Abalone were transferred to respirometry chambers attached to petri dish substrates to minimise handling. One experimental animal was assigned to an individual plastic 1.5 l respirometry chamber for data runs consisted of five abalone plus one blank (containing water but no abalone). One ml water samples were taken at regular intervals over a period of 2-4 h or until the water oxygen tension dropped to approximately 100 mmHg partial pressure of oxygen. Oxygen consumption was measured using an E101 oxygen electrode in conjunction with a Radiometer BMS Mk 2 blood gas analyser maintained within 0.5 °C of ambient respirometry chamber water, and connected to a PHM 71 Acid-Base Analyser (Radiometer, Copenhagen). The oxygen meter was calibrated using 2% sodium sulphite (zero PO_2) and air saturated seawater set to a PO_2 of 159 mmHg. Sample readings were taken after 3 min equilibration.

Trial 1 was conducted at 20 °C and trial 2 at 16 °C. Oxygen consumption was measured as reduction in mmHg PO_2, converted to μmol O_2 (Appendix 2, Cameron 1986) and expressed as μmol $O_2.g^{-1}$soft body weight.h^{-1}. Statistical comparison was made using Mann-Whitney U Test as data sets were small and underlying distributions unknown.

8.2.7 Ammonia excretion

Ammonia excretion rates were compared using the long-term spionid treatment cohort (August 1998 intake 1- Chapter 7.2) at Huon Aquaculture, sampled in October 2000. Ten heavily mud worm infested animals were compared to 10 less infested animals that were air-dried 10 months previously.

Mean length and weight of abalone were 63.6 ± 4.4 mm and 38.3 ± 7.1 g respectively. Mean blister coverage was 28.6% (SD= 8.8) for highly infested stock as compared to 10.9% (SD=7.9) for the less infested group. These differences, which could not be assessed until the completion of ammonia excretion measurements, were significant by Mann-Whitney U Test ($U=9.0$, $P<0.01$). Abalone were placed in 2 l aquaria with aeration at 13 °C for 6 h after which 50 ml samples were taken and frozen at –20 °C. Ammonia was measured using the salicylate-hypochlorite assay (Verdouw et al., 1978) against a range of standards read at 650 nm in a spectrophotometer. Ammonia excretion was expressed as μmole NH_4-N.g^{-1}.$h.^{-1}$ and statistical comparisons were made using the Mann-Whitney U Test.

8.2.8 Starvation comparison

As indicated in Chapter 7.3 one cohort of abalone with a severe mud worm infestation began to die after approximately 18 months of exposure. As underfeeding was also considered a factor in this outcome, a three-month starvation trial was conducted in an attempt to differentiate between the effects of mudworm infestation and starvation. A pool of 40 abalone, with a mean length of 44.6 ± 4.2 mm and mean weight of 12.7 ± 4.2 g was obtained from farm 1 (Chapter 2) in early February 2001. Abalone were tagged (Chapter 2), measured (length and weight), and housed in plastic aquaria within the Fish Health Unit recirculating system (Chapter 2). At the beginning of the time sequence and at 4 wk intervals thereafter for 12 weeks five animals were sampled, remeasured, Na^+/K^+ ratio determined and tissues were fixed for histology.

8.3 Results

8.3.1 Clinical pathology data

Testing of apparently healthy abalone populations was conducted to ascertain a "normal" range for apparently healthy farmed abalone and allow comparison with moderately and severely mud worm-infested stocks (Table 8.1). Only potassium ($P<0.05$, $t=2.98$, 18 df) and the Na^+/K^+ ratio ($P<0.001$, $t=-5.41$, 22 df) were significantly different (by two tailed t-test) between spionid infested and control stocks for those bled from the foot (Appendices 8A and 8B). Statistical analysis of the smaller group bled from the cephalic sinus bleed site showed no significant differences between mud worm infested and normal abalone ($p>0.05$ Mann-Whitney U Test) for any of the variables. Testing of water samples from the abalone culture sites found a mean Na^+/K^+ ratio of 44.4 (SD=0.5, n=5).

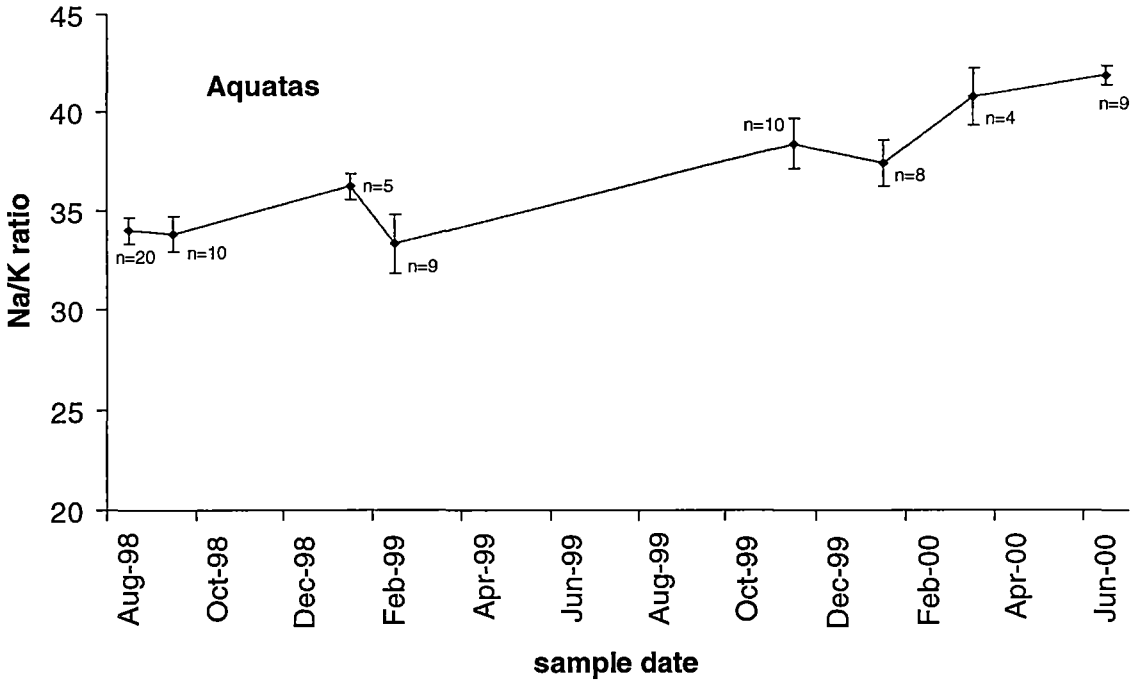
Sufficient samples for full testing of electrolytes were not consistently withdrawn from the cephalic sinus in abalone under 50 mm in length. Sampling of haemolymph from the foot of abalone less than 20 mm length was also difficult. Samples taken from 15 mm abalone (including composite samples from two or more animals) had a Na^+/K^+ ratio of 18.3 (SD=1.7, n=3), rising to 30.0 (SD=2.7, n=7) for 19 mm abalone and 32.2 (SD=2.7, n=5) for 28 mm abalone.

Sodium/potassium ratio values changed significantly with time at both Huon Aquaculture (df 8 69, $P<0.001$) and Aquatas (df 7 67, $P<0.001$). The initial control sample for both sites (August 1998) had a ratio of 34.0 (SE=0.65, n=20), while the final sample taken in October 2000 at Huon Aquaculture had risen to 36.6 (SE=0.5, n=10)(Figure 8.1). This was a lower rise than that of the equivalent cohort at Aquatas where the final two Na^+/K^+ ratio samples in March and June 2000 exceeded 40 (Figure 8.1). Aquatas stock of this cohort began dying in early 2000 (Chapter 7).

Table 8. 1 Haemolymph parameters from two bleed sites for normal and mud worm infested abalone. Means with SD and n values in parenthesis.

	normal abalone		spionid infested abalone	
units	foot	cephalic sinus	foot	cephalic sinus
Cu^{2+} ($\mu mol.l^{-1}$)	209.2 (27.5, 10)	213.7 (29.8, 6)	235.1 (100.5, 10)	337.0 (21.0, 5)
Cl^- ($mmol.l^{-1}$)	499.2 (30.7, 9)	508.4 (22.2, 6)	490.7 (44.6, 11)	503.2 (18.5, 5)
K^+ ($mmol.l^{-1}$)	13.5* (1.5, 11)	11.6 (0.6, 6)	11.9* (1.2, 13)	11.1 (0.6, 5)
Na^+ ($mmol.l^{-1}$)	463.5 (46.7, 11)	469.1 (31.0, 6)	465.0 (53.7, 13)	465.1 (19.7, 5)
Na^+/K^+ ratio	34.6* (2.1, 11)	40.6 (1.2, 6)	39.2* (2.0, 13)	41.8 (0.9, 5)
Ca^{2+} ($mmol.l^{-1}$)	10.6 (1.1, 8)	10.6 (0.9, 6)	10.7 (0.8,10)	11.0 (0.7, 5)
Mg^{2+} ($mmol.l^{-1}$)	49.8 (4.3, 8)	50.9 (4.0, 6)	47.4 (2.9, 10)	47.6 (5.7, 5)
Glucose ($mmol.l^{1}$)	0.4 (0.2, 4)		0.2 (0.2, 6)	
Protein ($g.l^{-1}$)	9.8 (0.4, 4)	9.3 (0.6, 2)	9.5 (1.8, 6)	11.1 (0.6, 3)

* site location pairs for row means are significantly different ($P<0.05$)



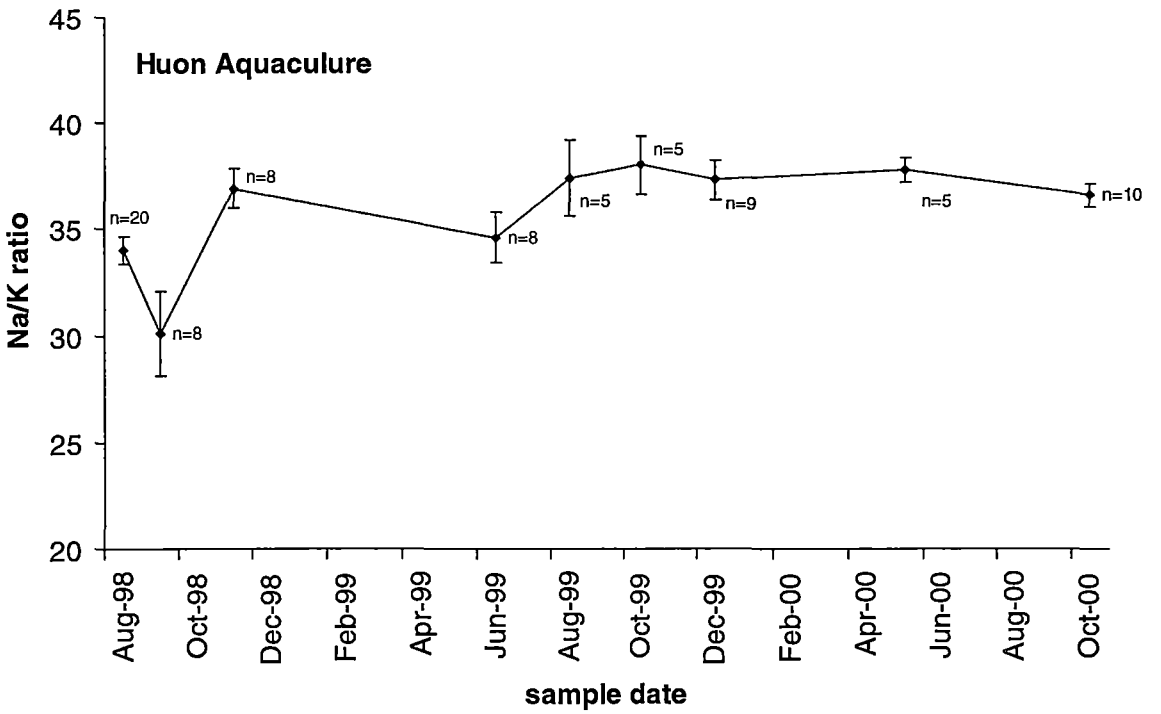


Figure 8.1 Temporal change in haemolymph sodium/potassium ratio for mud worm infested abalone at two study sites (means ± SE)

Air-dried and untreated abalone from the long-term treatment experiment (Chapter 7) were sampled four times: in June and December 1999 and in June and October 2000. Sodium/potassium ratio data were tested using two way ANOVA with treatment (dried/non-dried) and time as factors: time was significant ($P<0.05$) but treatment was not ($P>0.05$). The ANOVA table is located in Appendix 8C. Previous data indicated that it was not possible to prevent some spionid infestation and shell blistering of the treated group (Chapter 7).

There was no significant difference by Kruskal-Wallis test ($H_c = 6.154 < \chi^2_{0.05,3} = 7.815$, Appendix 8D) between Na^+/K^+ ratio data for monthly samples (Feb, Mar, Apr, May 2001) from experimentally starved abalone. The grand mean was 37.2 (SD=3.4, n=19).

8.3.2 Histology

Abalone infested in 1994-1996

All heavily mudworm infested stock showed elevated levels of brown staining pigment granules in the right kidney tubules (Figure 8.2). Low levels of fine pigment were sometimes present in spionid-free affected presumptive healthy control animals. Severely spionid infested abalone also showed reduced right kidney definition (Figure 8.2) and enlargement of the lumen (Figure 8.3) compared to controls (Figure 8.8). Large, brown pigmented granules were typically located in the tubules of the digestive gland or digestive diverticulum (Figure 8.5) and not seen in control animals. Enlargement of the digestive tubule lumen (Figure 8.6) and decrease in interstitial tissue (Figure 8.7) were also seen in severely mud worm infested abalone. Normal digestive gland structure is shown in Figure 8.8.

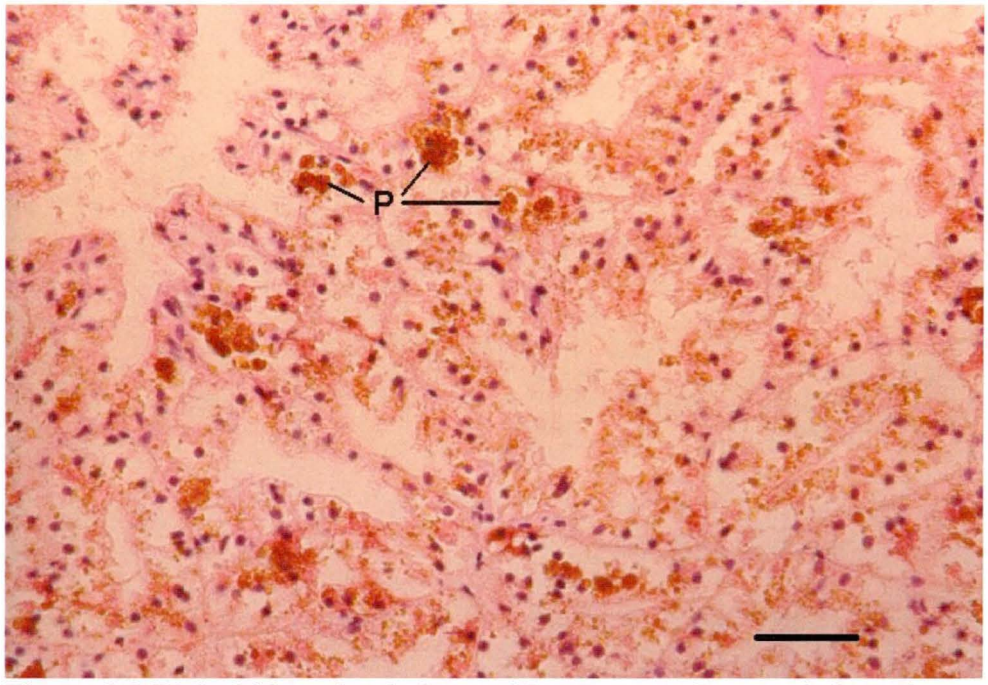


Figure 8.2 Right kidney in abalone with severe mud worm infestation. P = large pigment aggregations, bar = 100 μm

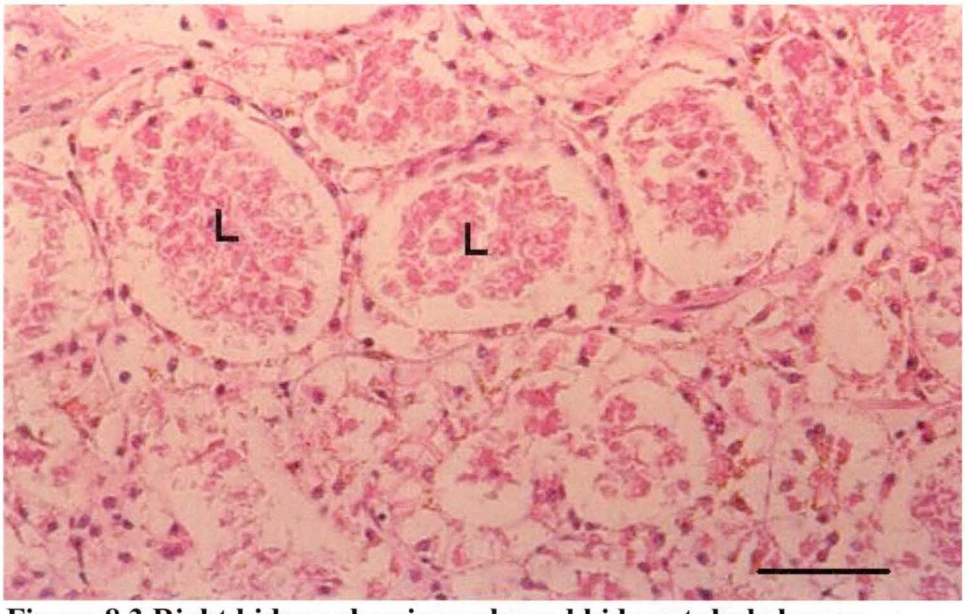


Figure 8.3 Right kidney showing enlarged kidney tubule lumen. L = enlarged lumen with sloughed cells, bar = 100 μm

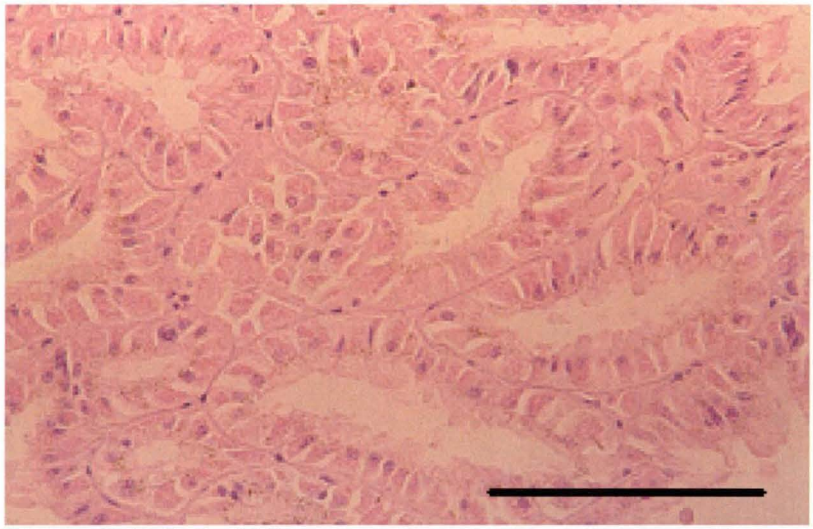


Figure 8.4 Right kidney structure in control, non-mud worm infested abalone, bar = 100 µm

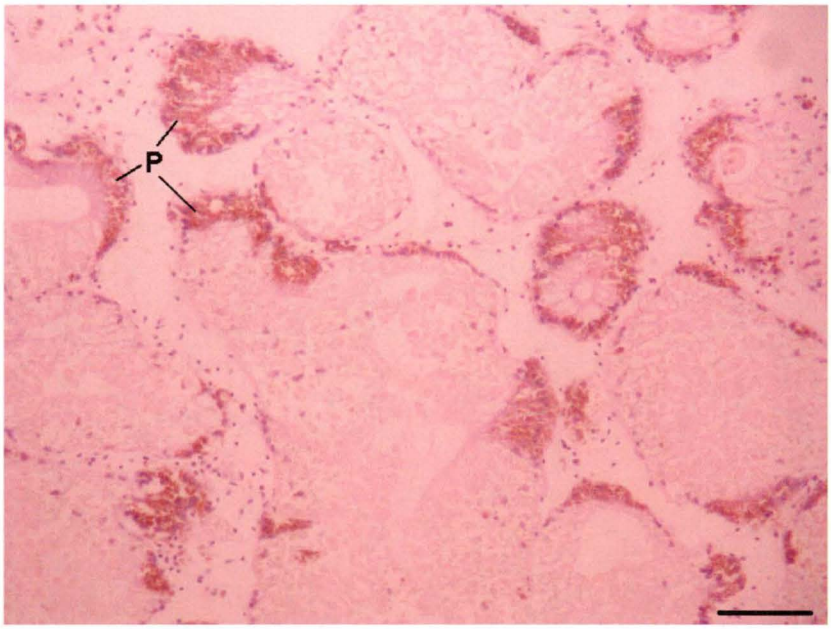


Figure 8.5 Digestive gland in severely mud worm infested abalone. P = pigment deposits, bar = 100 µm

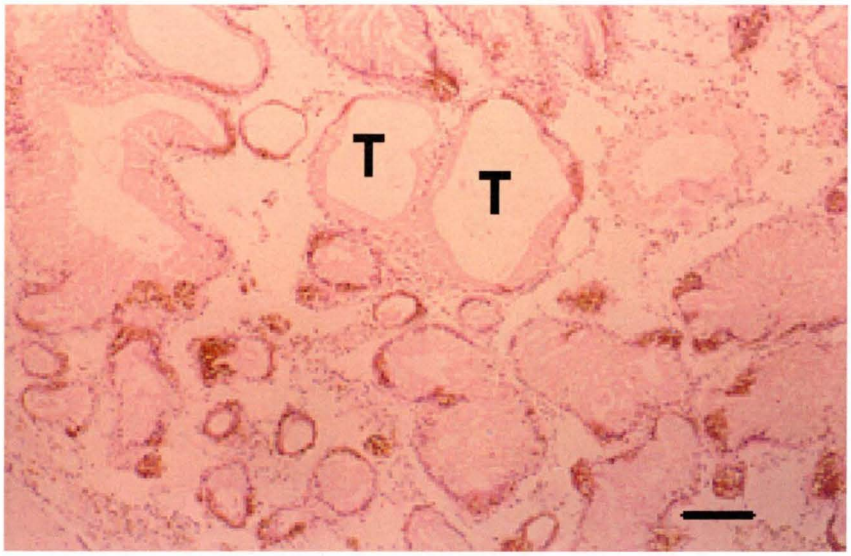


Figure 8.6 Digestive gland, severely mud worm infested abalone. T = tubules with enlarged lumens, bar = 100 μm

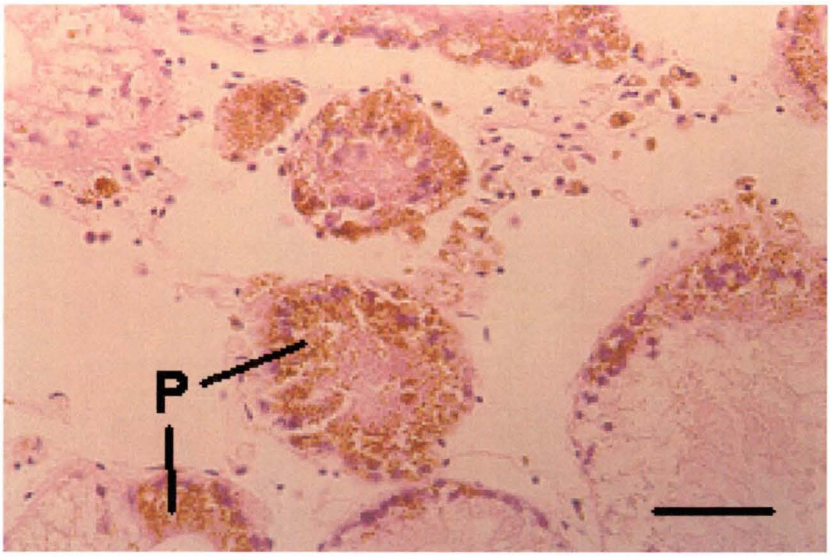


Figure 8.7 Close up of digestive tubules showing reduction in interstitial tissue between tubules and pigment deposits (P), bar = 100 μm

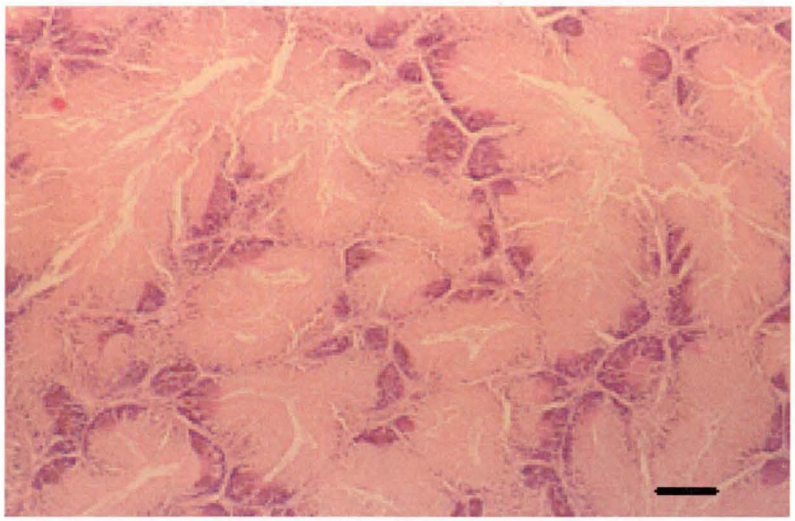


Figure 8.8 Normal digestive gland structure, bar = 100 μm

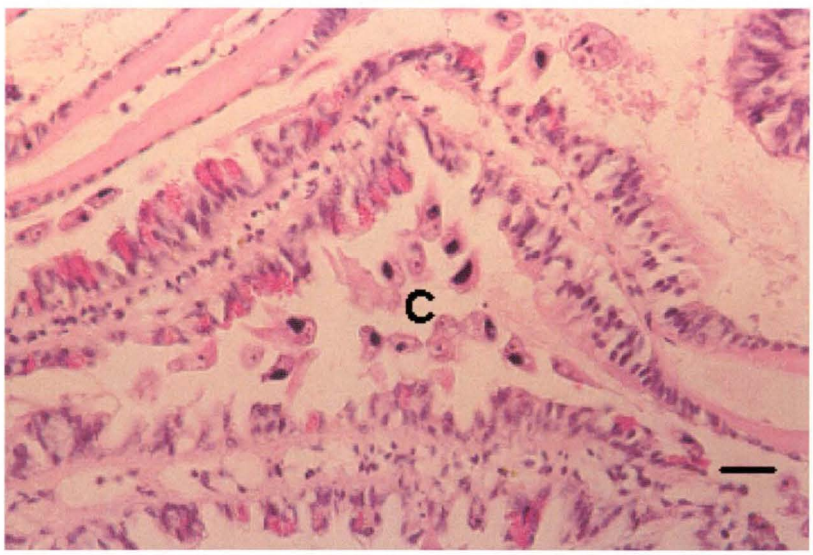


Figure 8.9 Gill ciliates (C) in mud worm infested abalone, bar = 25 μm

Epithelial cells of the stomach and intestine also showed the presence of brown pigment, to a greater extent than normal controls. A lack of food was often observed in the digestive systems of severely spionid infested abalone.

There appeared no consistent changes in gill structure of mud worm infested abalone and ciliates were present (Figure 8.9). Sections of foot tissue stained with PAS showed reduced PAS positive glycogen tissue (Figure 8.10) contrasting with normal abalone (Figure 8.11). Glycogen tissue was also depleted in the digestive tubules of mud worm infested stock (Figure 8.12) compared to healthy abalone (Figure 8.13). There was no evidence of increased haemocyte activity or lesions in the shell muscle adjacent to blistered shell. Nor was there obvious or consistent depletion of muscle mass seen by H&E or specific Martius Scarlet Blue stain for muscle fibres.

Histology: August 1998 Intake

Animals in this time cohort were at least three years old in mid-1998 and were considered relatively slowly growing. Histology of abalone collected May 1998 before transfer of animals to the sea showed no pigment in the right kidney. Further histology on abalone collected August 1998 during transfer to sea-based farms showed the presence of grade 1 right kidney pigment in 3/5 animals and grade 1 pigment in the digestive tubules of one specimen. Two animals also had pigment in the epithelial cells of the intestine. In abalone from Huon Aquaculture, moderate to heavy (grades 2 and 3, respectively) quantities of brown pigment in the right kidney became progressively more common in the two years following transferral to this site (Figure 8.14). Conversely, the final sample taken in October 2000 showed a return to the minimal kidney pigmentation observed at the beginning of the time sequence. The extreme levels of pigment seen previously in abalone surviving the first reported mud worm outbreaks were not recorded. Reduction in right kidney definition and enlargement of the lumen were rarely seen.

Brown pigment in the digestive tubules was absent during the first year of sampling, with some scores of "1" and "2" recorded after October 1999.

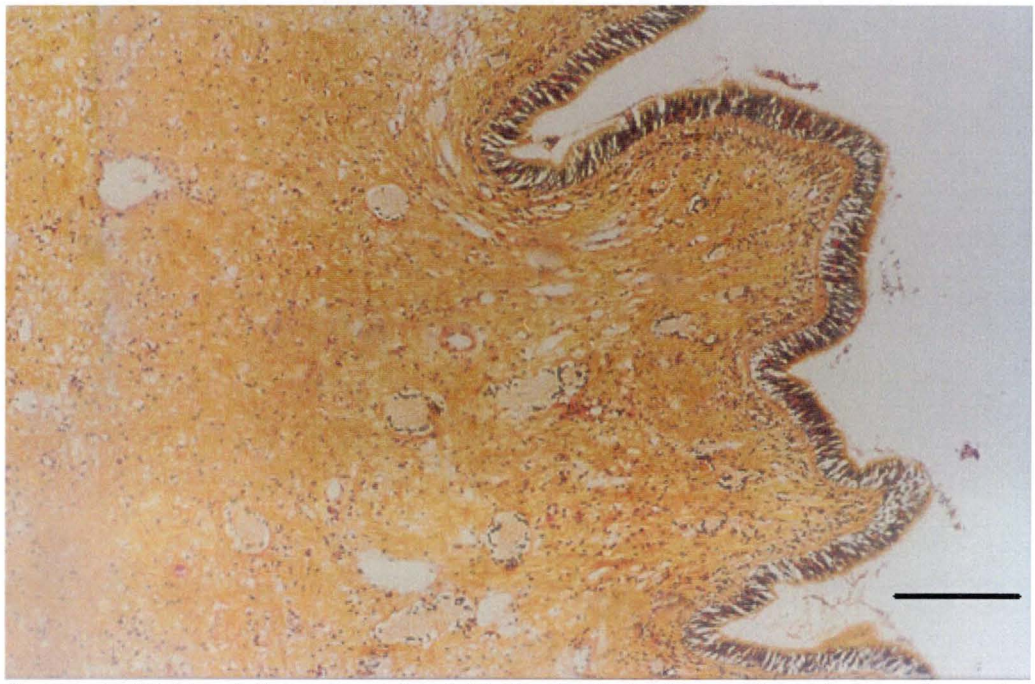


Figure 8.10 Foot tissue, spionid infested abalone showing reduced PAS +ve (red staining) glycogen tissue, bar = 100 µm PAS stain.

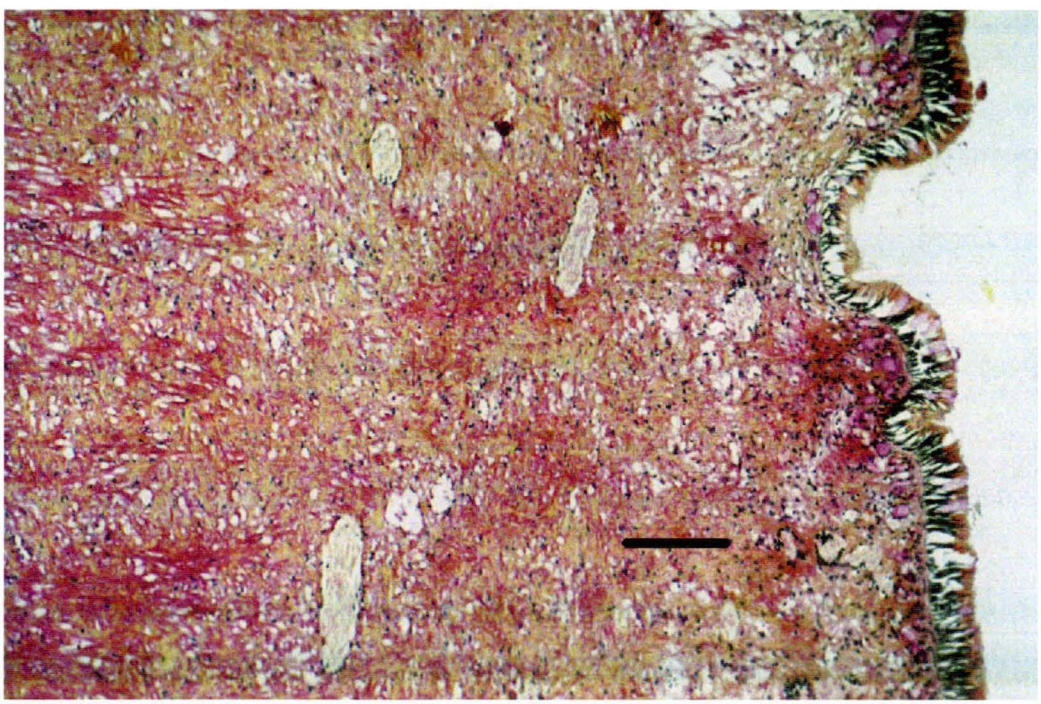


Figure 8.11 Foot tissue, normal abalone showing glycogen (red staining) tissue, bar = 100 µm PAS stain.

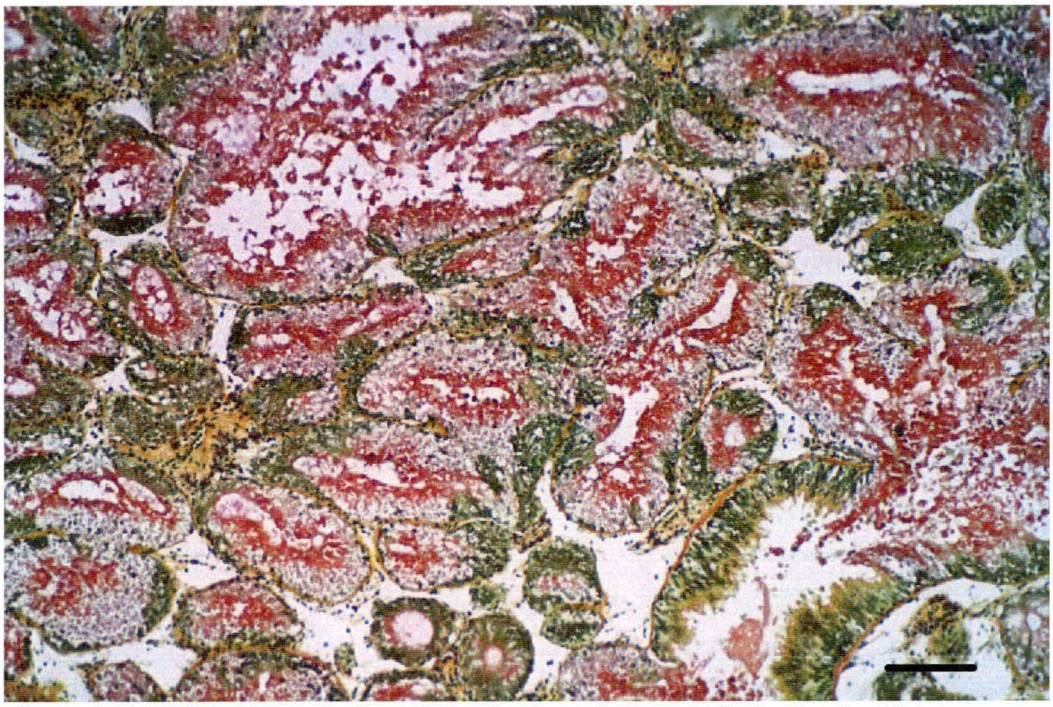


Figure 8.12 Digestive tubules of mud worm infested abalone showing reduced glycogen tissue (red), bar = 100 µm. PAS stained section.

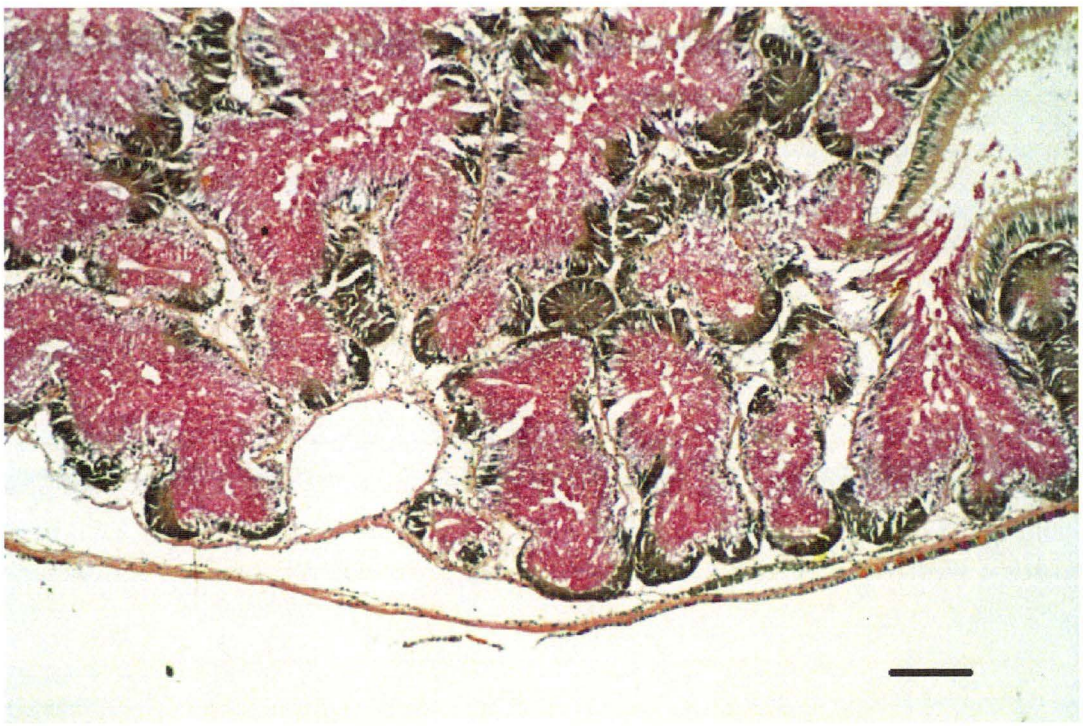


Figure 8.13 Normal digestive gland tissue showing glycogen storage areas (red), bar = 100 µm. PAS stain.

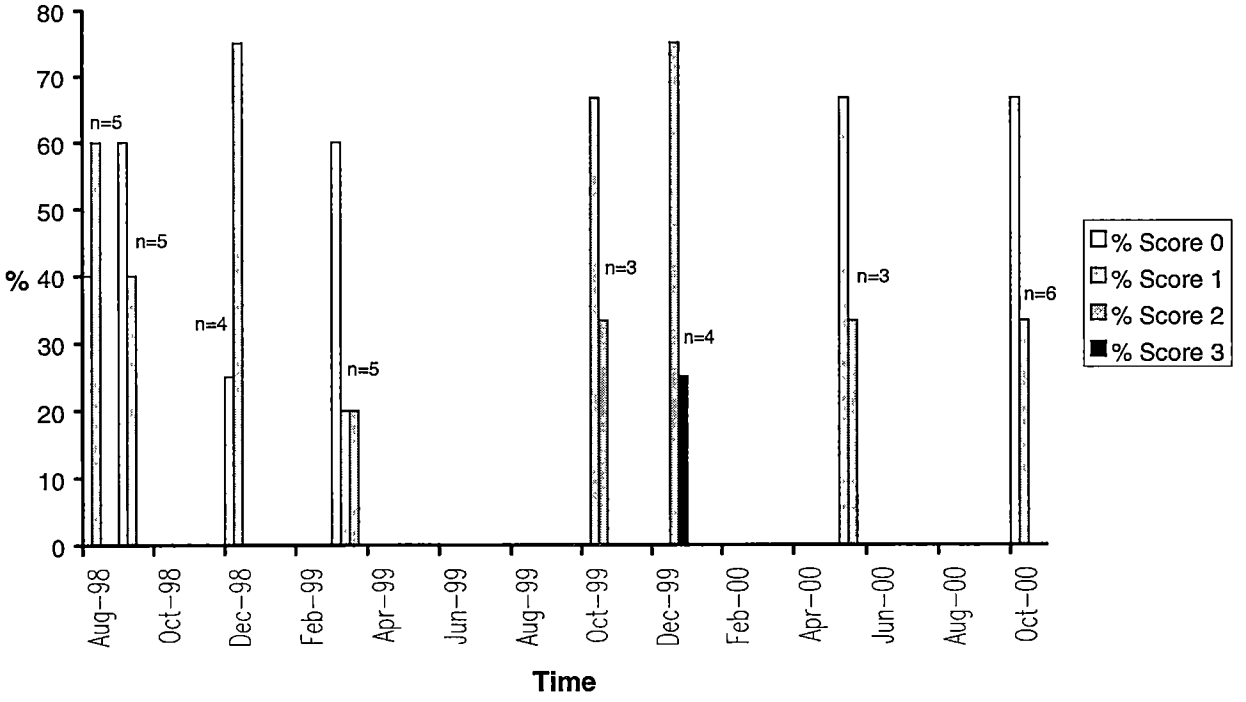


Figure 8.14 Temporal change in subjective right kidney pigment score for August 1998 intake abalone at Huon Aquaculture

Moderate reduction in digestive tubule height and interstitial tissue was associated with light to moderate digestive tubule pigmentation in abalone sampled December 1999 and later. Pigmentation in the gut epithelium was widespread in all abalone sampled June 2000. Final samples taken October 2000 showed the presence of gill ciliates in four of seven abalone. Gonad development was observed in samples taken summer 1999 and 2000. There was insufficient material to determine whether the heavy mud worm infestation present by late 2000 suppressed sexual maturity.

The equivalent August 1998 stock transfer to Aquatas developed grade "2" pigment in the right kidney by September 1999 as well as some digestive tubule pigment. By March 2000 when many of the animals had died, scores of 2 and 3 were recorded in the right kidney of remaining specimens. The last sample taken in June 2000 was characterised by poor right kidney definition including the presence of vacuoles and dilation of many tubules. Fine brown pigment was widespread but only rated "1" in four abalone examined. There was a reduction of interstitial tissue between digestive tubules in all samples, and a decrease in digestive tubule height in three of four samples. Pigment in the digestive gland was rated 1-2. Other than spionid infestation there were no significant pathological changes of abalone tissue or shells.

Spring 1998 Intake

Farm 1 controls sampled September 1998 had little brown pigment in the right kidney, digestive gland tubules or gut epithelium. Digestive tubule height was normal with all "0" ratings; a reduction in interstitial tissue was observed in one specimen. Additional controls sampled January 1999 had a common finding of grade "1" pigment in right kidney tissue although not in digestive tubules and gut epithelium. Digestive tubule height remained normal with all specimens recording ratings of zero.

At the Aquatas site, abalone had developed a consistent finding of grade "1" pigment rating in the right kidney when sampled in September 1999 (after a year at the site), but with minimal mudworm infestation. Grade 1 scores for pigment were

recorded in the digestive tubules for some specimens and in the gut epithelium. This trend continued for abalone sampled in June 2000. Growth of abalone in this cohort was poor and mud worm blistering was not considered serious with a mean of 5.1% (SD= 5.7%, n=10) in June 2000.

At Huon Aquaculture abalone sampled in September 1999 after a year at the site all recorded at least grade 1 right kidney pigment with some pigment in epithelial cells of the intestine. Interstitial tissue between digestive tubules was reduced in many of the animals sampled at this time. A further sample taken in June 2000 showed consistent grade 1 right kidney pigment and intestinal pigment in 1 specimen. The height of digestive tubules was slightly reduced (grade 1) in one specimen; all specimens were free from pigment in this location.

At final sampling in January 2001 right kidney and digestive tubules showed good definition and no tubule dilation. Pigment was present in right kidney tissue of all four abalone sampled but stained grey rather than the previously observed brown colour. Similar pigment granules were found, to a lesser extent, in the digestive tubules. Gill ciliates were present in one abalone. Mud worm blistering was minimal in this stock cohort.

Three month experimental starvation of abalone resulted in few morphological changes to tissues during the first 8 weeks. Compared to initial control animals, abalone starved for 8 weeks showed no increase in amount of right kidney pigment present, no increased thinning of digestive tubules, no deposition of pigment granules in digestive tubules and no obvious decrease in foot muscle protein. Following 12 weeks of starvation a reduction in digestive tubule height and/or digestive gland interstitial tissue was observed in 3/5 abalone. Deposition of pigment granules in digestive tubules was not observed. Right kidney structure did not change markedly between starved and control animals. Fine but widespread brown pigment deposition was seen in the kidney of 1/5 12 week starved abalone. Foot tissue sections of this group stained with PAS showed a reduction in glycogen tissue.

8.3.3 Blister environment and microflora

All 10 blisters tested had pH in the range 7.5-8.0 as indicated by test strips (range 4.5-10.0). Water withdrawn from blister cavities and cultured on plate media (Fish Health Unit, Launceston) showed no known pathogens and bacteria were not considered present in large quantities by subjective assessment (Wagner 1999 pers. Comm.). Mixed *Vibrio* species were present and two of these were identified as *V. splendicus* and *V. aestuarianus.*

Histological sections of severely mud worm infested shells from a variety of sites showed no evidence of fungal hyphae in PAS and H&E stained sections. Similarly, no evidence of fungi was observed in soft tissues adjacent to blisters or elsewhere in infested abalone.

8.3.4 Tissue chemistry

Initial mean protein level in foot tissue was 2.98% (SE=0.53, n=5) wet weight in September 1998, 1 month post transfer. As abalone became spionid infested, protein levels declined by as much as 50% of initial values (Table 8.2). The lower levels were comparable to data for experimentally starved abalone (Section 8.4 – Discussion). Statistical analysis is given in Appendices 8E and 8F.

Table 8.2 Temporal variations in foot tissue protein content with mud worm infestation at two study sites (means ± SE).

Huon Aquaculture		Aquatas	
Date	% protein	Date	% protein
Sep 1998	$2.98^{AB} \pm 0.53$, n=5	Sep 1998	$2.98^{A} \pm 0.53$, n=5
Dec 1998	$1.81^{BCD} \pm 0.01$, n=4	Feb 1999	$1.01^{AB} \pm 0.03$, n=5
Dec 1999	$1.68^{BC} \pm 0.14$, n=7	Nov 1999	$0.79^{B} \pm 0.08$, n=5
May 2000	$1.59^{C} \pm 0.29$, n=4	Jun 2000	$1.25^{AB} \pm 0.21$, n=5
Oct 2000	$2.58^{AD} \pm 0.20$ n=13		

Column means with shared superscripts are not significantly different (P>0.05)

8.3.5 Respiration rate

Mean oxygen consumption was 1.50 ± 0.19 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=8) in chronically spionid-infested stock at 20 °C, significantly higher (U=3.0, P=0.01 Mann-Whitney U Test), than that of uninfested control abalone 0.81 ± 0.15 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=4). Dissection of animals revealed that percentage flesh weight (Chapter 7.2.5) of mud worm infested stock was 60.3% ± 2.3%, as compared to 82.3% ± 4.4% for uninfested stock ($\overline{X}$ ± SE, n=8 and 4 respectively) a statistically significant difference (U=0, P<0.01 Mann-Whitney U Test). In a second experiment at 16 °C, mean oxygen consumption was 0.96 ±0.05 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=5) in heavily spionid infested abalone, significantly higher (U=0.0, P=0.01 Mann-Whitney U Test) than that of lesser (methods: section 8.2.6) mud worm affected animals 0.68 ±0.04 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=5).

8.3.6 Ammonia excretion

The ammonia excretion rate of extensively blistered abalone was 0.29 ± 0.08 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE=, n=10), not significantly higher (U=44, P>0.05, Mann-Whitney U Test) than that of lesser affected animals 0.21 ± 0.04 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=10). The less blistered abalone were intended to be a non-spionid infested control but post experiment dissection revealed blister coverage of 10.9% compared to 28.6% for the highly infested animals (section 8.2.7).

8.3.7 Starvation Trial

Sixteen of 40 abalone died in the week following transport and the commencement of the trial period. Clearly these deaths could not be assigned to starvation but appeared to be due to transport stress. An additional abalone died 8 weeks following the commencement of the trial.

Following 12 weeks of starvation abalone had a mean weight loss of 11.9% (SD=6.5%, n=6). Histological and clinical pathology findings are discussed in the appropriate sections above.

8.4 Discussion

The haemolymph of marine molluscs (which corresponds to the blood and interstitial fluid of higher animals) is very similar in ionic composition to the surrounding seawater (Burton 1983). Solute haemolymph concentrations of potassium, sodium and chloride were similar between this study and that of Harris (1999) using greenlip abalone. Calcium and magnesium levels were somewhat higher in this study than that of Harris (1999) and both sodium and chloride levels were about 50 $mmol.l^{-1}$ higher than those reported from greenlip abalone by Boarder (1997). These studies in total comprise a useful data base of normal blood parameters that may be useful in determining health status of abalone generally.

While there is little, if any, regulation of sodium, chloride and magnesium in marine molluscs, potassium concentration in haemolymph may exceed that of seawater by a factor of 1.1-1.5 (Burton, 1983). Potassium levels in apparently healthy abalone from this study averaged 13.5 and 11.6 $mmol^{-1}$ for foot and cephalic sinus sample sites, respectively (Table 8.1, ionic composition). These are elevated compared to mean potassium concentration of 9.7 $mmol^{-1}$ for seawater from southern marine farms (Appendix 8G) and, thus, consistent with Burton (1983). Interestingly, while potassium levels in healthy abalone were regulated at higher than seawater concentrations, these levels fell, significantly so, in samples drawn from the foot of mud-worm infested animals (Table 8.1). As noted above, potassium levels in haemolymph were greater in samples withdrawn from the foot than from the cephalic sinus of abalone. Also, the magnitude of the decrease in potassium levels, between apparently healthy and moderately-severely spionid infested animals, was greater in samples withdrawn from the foot than the cephalic sinus. In part, this may be a consequence of potassium leakage from cells damaged as a result of the foot sample method (Chapter 2). Russell and Evans (1989) report that several

authors describe means by which the foot can be isolated from the rest of the abalone cardiovascular system. If this is true it may be that mud worm stressed abalone attempt to maintain osmoregulation in the visceral mass at the expense of the foot – leading to a greater drop in haemolymph potassium from this site relative to the cephalic sinus. Whatever the cause, potassium appears to be regulated in abalone and is decreased in mud worm infested animals. As the magnitude of potassium variation was greatest in samples from the foot, this site may possibly provide a more sensitive measure of stress than the cephalic sinus. Potentially, testing of haemolymph potassium levels may have some application as a general stress test in abalone.

Testing of haemolymph copper found no significant differences between moderately to severely mud worm infested abalone and apparently healthy animals from either bleed site. The foot bleed data set for worm infested stock was highly variable (Table 8.1). Such variation was noted in apparently healthy farmed abalone by Harris (1999) and in field studies by Ainslie (1980). Therefore, assessment of respiratory pigment level by indirect copper measurement appears of little value as health indicator. Possibly, direct measurement of the abalone respiratory pigment haemocyanin is worth investigating.

Haemolymph glucose levels were approximately twice as high in apparently healthy as compared to mud worm infested abalone (Table 8.1), however; this was not statistically significant ($P>0.05$, Appendix 8B). A reduction in glucose levels in spionid infested stock would not be unexpected given the respiration data (section 8.3.5) indicated an increase in metabolic rate for these abalone. Cheng and Lee (1971) and Livingstone and De Zwaan (1983) reported significantly lower glucose levels in the haemolymph of parasitised molluscs. The mean level of 0.2 $mmol^{-1}$ glucose in the infested abalone in the present study was very similar to the levels reported for reduced salinity stressed abalone by Boarder (1997). Normal levels of haemolymph glucose were higher in the study by Boarder (1997) compared to the present study (0.75 compared to 0.40). Sample collection trips were conducted over 2-3 days in the present study followed by relocation to the laboratory before processing. Abalone were not fed in this time which would be sufficient, based on

data presented by Carefoot et al., (1993), for glucose levels to drop markedly. For this reason haemolymph glucose sampling was not considered a high priority. It may, however, be worthy of further investigation as an indicator of abalone health, especially where stock are still feeding.

There were no significant differences between haemolymph protein levels in mud worm infested and normal stock. This is consistent with the copper data, as most, if not all, protein in the haemolymph would be expected to be associated with the copper bound haemocyanin respiratory pigment. Lee and Cheng (1972) as cited by Malek and Cheng (1974) found that haemolymph protein reduction in the planorbid snail *Biomphalaria glabrata* as a result of parasitism with *Schistosoma mansoni* was largely due to reduction in the respiratory pigment (haemoglobin) concentration.

Histological examination was performed on abalone that survived the initial mud worm outbreaks in the mid-1990's and on a sequence of increasingly infested stock placed at the study sites in 1998. Major findings involved changes to the right kidney and digestive tubules, including substantial deposition of brown pigment granules. The reduction in right kidney definition and enlarged lumen associated with moderate and severe mud worm infestation has been reported by Harris et al. (1998) in relation to ammonia exposure. Brown pigment granules were described in the cytoplasm of normal abalone right kidney cells (Bevelander, 1988) and the elevated quantities associated with moderate and severe mud worm infestation have been previously reported by Harris et al (1998) in relation to elevated nitrite exposure. These authors suggested increased pigment might be a reflection of increased kidney protein and, hence, cell turnover. Malek and Cheng (1974) note that yellow/brown "excretory or ferment globules" are a feature of parasitised molluscan digestive glands particularly when the host-parasite interaction is of long duration. These authors state that the globules represent accumulated metabolic wastes in the digestive gland. The pigment granules in the two tissue types, therefore, indicate a long-term stress consistent with length and severity of mud worm infestation in the present study.

Mud worm infestation apparently led to depletion of host energy reserves. During starvation glycogen, triglyceride and protein reserves are utilised consecutively (Takami et al., 1995). This is similar to the progression of withering syndrome of abalone in California (caused by a rickettsia) in which morphological changes result in the digestive diverticula and carbohydrate storage is reduced in terminal secretory tubules of infected animals (Gardner et al., 1995). This leads to foot muscle atrophy through prolonged starvation. Similarly, PAS staining of severely mud worm infested abalone showed reduced carbohydrate stores in the digestive gland and routinely stained sections showed increased tubule lumen size - also indicative of poor nutrition. The foot of severely mud worm infested abalone was sometimes completing lacking in glycogen tissue. However, the degree of foot atrophy described by Gardner et al. (1995), including severe depletion of muscle fibres was not seen in the present study. Examination of tissue sections of severely spionid infested abalone revealed no evidence of rickettsial infection associated with withering syndrome.

Normal levels of soluble protein in the foot of blacklip abalone were similar to those reported by Takami et al. (1995) for *H. discus.* The reduction in protein to less than about 1.5% wet weight in mud worm infested stock was similar to levels in abalone starved for 30 day (Takami et al., 1995). Protein levels in some infested stock from Aquatas (Table 8.2) were like those of 70 day starved stock reported by Takami et al. (1995). Interestingly, foot protein levels in mud worm infested stock at Huon Aquaculture recovered from low values to close to the normal level in the final October 2000 sample (Table 8.2). This foot protein recovery coincided with an absence of moderate pigment deposits in the right kidney. The kidney pigment had been present in most samples taken in the previous year and the finding suggests that the right kidney pigment was associated with catabolism of foot protein.

Respiration rates in this study are consistent with those quantified in previous studies on abalone by Uki and Kikuchi (1975), Barkai and Griffiths (1987), Nimura and Yamakawa (1989), Segawa (1991), Carefoot et al., (1993), Paul and Paul (1998) and Harris (1999) – refer to Appendix 8H for comparisons. Studies on heart rate and respiration of the gastropod *B. glabrata* parasitised by the larval trematode

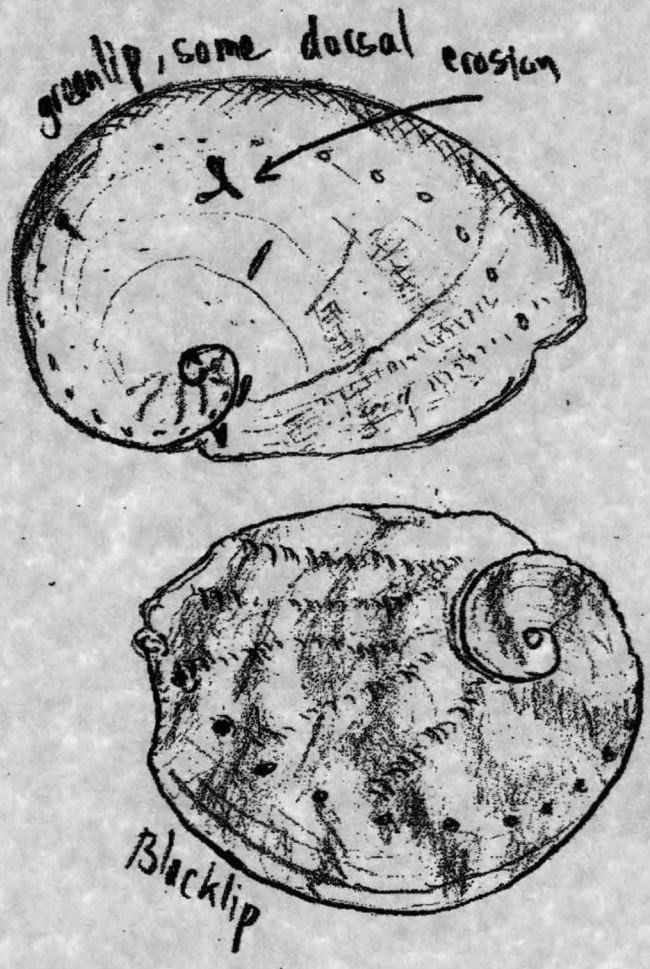
S. mansoni have generally showed an increase in infested stock relative to controls (Malek and Cheng, 1974). This was consistent with the two respiration trials in the present study but contrasted with oxygen consumption data for abalone with withering syndrome where consumption decreased (Kismohandaka et al., 1993) or remained unchanged (Kismohandaka et al., 1995) relative to unaffected animals. Possibly the interaction between the disease and extent of host starvation could affect respiration data. It has been shown by Segawa (1991) and Gaty and Wilson (1986) that oxygen consumption in abalone decreased in starved animals.

There was no significant difference between ammonia excretion for greater and lesser mud worm infested stock. Excretion rates were similar to those of abalone of comparable size reported by Segawa (1991) for *H. diversicolor* but higher than excretion rates for *H. midae* (Barkai and Griffiths, 1987). There is little literature relating ammonia excretion in abalone or other molluscs to disease. Kismohandaka et al. (1993) found that abalone with withering syndrome excreted 3.8 times more ammonia per gram wet weight than did healthy animals. The contrast between this study and the mud worm infestation data may be due to the lack of a suitable mud worm free control in the latter case. Also, as for oxygen consumption data, the extent of starvation associated with the disease condition might influence the outcome. Segawa (1991) found that ammonia excretion at first increased in starved abalone then decreased after 17 d.

The changes that occurred in abalone that contracted mud worm infestation as the result of experimental placement in 1998 were not as great as those that occurred when abalone were first farmed in susceptible areas. Stock first infested in 1994-96 and examined 1996-1999 had greater subjective shell damage ratings, decreased percentage flesh weight, increased *B. knoxi* counts and more extreme histological change compared to abalone in subsequent experiments. These changes manifested themselves in high mortality rates at a number of farms in the mid to late-1990's. Possible reasons for different levels of infestation and spionid impacts between past and present are examined in Chapter 9.

8.5 Conclusions

Moderate to severe mud worm infestation lead to depletion of abalone energy reserves. This was indicated by histological changes to the right kidney and digestive tubules consistent with increased cell turnover; with depletion of glycogen and pedal protein reserves; reduction in haemolymph glucose and with increased oxygen consumption. Such changes were likely the result of mobilisation and direction of host resources to shell repair, that may have manifested itself as reduced growth and, in some instances, death. Spionid infestation was also associated with reduced capacity to regulate potassium.


grandlip, some dorsal erosion
Blacklip

Frontispiece: greenlip and blacklip abalone shells

Chapter 9
GENERAL DISCUSSION, LIMITATIONS AND FUTURE DIRECTIONS

Mass mortality of sea-farmed abalone in southern Tasmania during the mid 1990's was linked to spionid mud worm infestation and led to this research. A multi-pronged approach was devised with the aim of producing an integrated package of measures to minimize the impact of spionids on abalone culture in these susceptible locations. The original culture situation was replicated as far as possible between mid 1998 and early 2001. In excess of 9000 abalone were placed at two locations with a previous history of severe spionid infestations. Interestingly only 200-300 of this stock became infested to the levels previously associated with abalone mortality. This indicates that the risk of severe mud worm problems are not as great as previously feared by growers on the basis of events in 1995-1996 (Chapter 1).

The relative lack of severe infestation in stock, specifically managed to maximise infestations for study, was a positive outcome for industry. However, a larger number of infested stocks would have allowed greater exploration of numerical relationships between spionid numbers and production measures such as growth and mortality rates and abalone condition indices. This limitation could possibly be overcome in future work by choosing at risk stock (e.g. larger, heavily spirorbid fouled animals) for field studies, or by attempting to introduce infestation in a controlled laboratory situation.

The research was intended to focus on the spionid species *B. knoxi*. This was because the species had not been previously recorded in Australia and because *B. knoxi* was numerically dominant at the farm with the greatest stock losses (Chapter 1). Unexpectedly, *P. hoplura* was the most numerous species present in abalone during this field research, outnumbering *B. knoxi* by 5-10 to 1 in heavily infested stock (Chapter 7). One affect of this outcome was to compromise field experiments devised to directly test the consequences of spionid infestation against otherwise identical uninfested control abalone. It was planned to conduct such experiments by air drying a number of *B. knoxi* infested stock to serve as controls.

This was done successfully, but because of differences in timing of larval dispersal between these species, such stock later become unexpectedly infested with *P. hoplura* forcing comparisons between "more" and "less" infested stock instead.

The mortality rate in this study was low and was confined to mud worm-infested, underfed stock at one site. This contrasts markedly with events in the mid 1990's where considerable mortality occurred at several locations (Chapter 1). Explanations for the difference in mortality rates between past events and the present study are of considerable interest to growers. There appear to be two major possibilities: that either during the past mortality episodes there were other important stress factors operating in addition to mud worm infestation, or that infestation was more severe in the past due to the nature of the infestation pattern at that time as discussed below.

As noted previously, there was mortality of the underfed, spionid-infested stock relative to the better managed cohorts in the study. This suggests the potential for adverse conditions to magnify the impact of spionid infestation. However, at least one grower has indicated that stocks were properly maintained with regard to feeding before and during original mud worm outbreaks. Further, Hindrum (1996) reported growth rates of 80-100 $\mu m.day^{-1}$ (not typical of underfeeding) in sea farmed abalone that later experienced heavy mortality rates.

Retrospective shell grading of abalone submitted from Huon Aquaculture in 1997 and used for treatment trial experiments (Chapter 4) showed that 16% had slight or no mud worm infestation (SSDR grades 0-1, Appendix 1B). Shells examined from this source in 1997 sometimes included high proportions of shells from dead abalone and at least some of these shells had little or no mud worm infestation. It is possible, therefore, that the most severely affected abalone, (grade 3 rated shells account for 36% of the 1997 sample- Appendix 1B), died largely as a result of their spionid infestation. Subsequently, the resulting poor water quality, in combination with some degree of mud worm infestation, may have caused the deaths of further stock. Available farm mortality data (Appendix 1A) for 1994-96 shows a high death rate in the Austral summer for 1995/1996. Another factor of potential importance was the widespread use in the mid-1990's of "tube"- type

rearing vessels. These had relatively restricted water flow, especially once fouled with seaweed after some time in the water (Chapters 2 & 6). Thus, the combination of mud worm infestation, summer water temperature and the presence of dead stock in a small, relatively sheltered containment vessel could potentially magnify the impact of severe mud worm infestation.

In trying to reconstruct factors that may have lead to the mass mortality of the mid 1990's the role of spionid species must be examined. As noted previously, (Chapters 1,7) *B. knoxi* was the dominant species present at the Huon Aquaculture Company during the original infestations, contrasting with its representation at both study sites during field trials. As this species was not previously recorded in Tasmania (Wilson et al., 1993) it was speculated that it may have been a recent and relatively harmful spionid introduction from New Zealand (Chapter 1). In New Zealand the species creates a shallow burrow within the outer shell layer (periostracum) of *H. iris* (Read 1975, Handley 2000) but is not noted for blister production.

It should be recalled, however, that infested abalone at sea based operations other than Huon Aquaculture (Chapter 1) showed the presence of both *B. knoxi* and *P. hoplura*. Furthermore, in the early days of land-based abalone farming one such farm in each of Tasmania and South Australia suffered stock mortality associated purely with *P. hoplura* infestation. Since that time verbal reports of abalone mortality associated with spionids other than *B. knoxi* have been made (Chapter 7). Thus, it is difficult to make a convincing case that *B. knoxi* is an unusually destructive spionid species.

Anecdotal evidence from Tasmanian oyster farmers that "there are good and bad mud worm years" is supported by Pacific oyster blister data collected by the Fish Health Laboratory (Appendix 1C). This data gives the percentage of blister positive oysters in farmed populations but not the extent of blistering or the spionid species responsible. None the less, it is interesting that the highest blistering levels were recorded in 1995 based on this data.. Available abalone mortality data shows death rates of 12% and 32% between December 1995 and April 1996 for animals stocked in January and August 1995, respectively.

Stock placed in June 1994 suffered little mortality until December 1995, with an overall death rate of 31% by April 1996 (Appendix 1, Figure 1). These farm mortality data are consistent with the growth trial studies of Hindrum (1996) which reported 40% abalone mortality between November 1995 and April 1996.

These records support the possibility of heavy *B. knoxi* settlement at this site in the spring of 1995. Most of the abalone for which temporal mortality data are available were placed at 20-30 mm. This contrasts with the present study, in which stock placed at between 20-40 mm were minimally infested, in line with experiments on stock size and mud worm settlement (Chapter 6), and suggests relatively high numbers of larvae were present in the water during spring 1995. The rainfall data for Dover (Appendix 9A) near Huon Aquaculture are of interest in relation to factors that might contribute to spionid infestation. July and August 1995, just before the presumptive *B. knoxi* larval dispersion phase of 1995, received approximately twice the 100 year average rainfall for these months. Calculation showed that rainfall for July and August of that year, plus during the spring months when the *B. knoxi* is in the water column, was the third highest in 100 years (Appendix 9A). Runoff from heavy rain could benefit spionids by putting more nutrients in rivers and the sea leading to enhanced production of spionid larval food sources. This is consistent with references to increased spionid populations near drains and other sources of organic pollution (Chapter 6.6).

The rate of mud worm recruitment rather than the numbers per se may be important determinants of host impact. Abalone from Huon Aquaculture, placed 1994-1995 and surviving until 1997 had up to 40-50 *B. knoxi* chimneys (Chapter 2). It is plausible, therefore, that stock deceased before this time had even higher levels of infestation. Total spionid numbers in the August 1998 Huon Aquaculture experimental intake abalone eventually reached levels seen in the 1997 survivors but this took approximately 2 years (Chapter 7). If, by contrast, in spring 1995 the Huon Aquaculture Company abalone experienced settlement of, for example >30 *B. knoxi*, the effects might have been sufficiently devastating to explain the onset of mortality in December 1995 - April 1996.

In the present research, the reproduction of the two major spionids, *B. knoxi* and *P. hoplura*, was investigated with an emphasis on the possibility of avoiding larval settlement. *Boccardia knoxi* was found to have an exclusively planktotrophic larval development confined to the Austral spring, consistent with the findings of Handley (2000) for *B. knoxi* in New Zealand. The reproductive strategy behind planktotrophy is one of high larval production (estimated at ~700 larvae/brood - Chapter 3) to account for a high attrition rate. There is also considerable scope for dispersion, due to the length of time in the plankton. This "boom or bust" capacity for larval production may account for both the original mortality episodes as described above and the relative scarcity of the species 1998-2001. Thus, *B. knoxi* (and other planktotrophic spionids) may have the potential to cause significant harm to mollusc culture enterprises when environmental conditions favour high larval survival.

The consistency of *B. knoxi* seasonal reproduction (Chapter 3) fortuitously allows a simple avoidance strategy to be practiced in susceptible areas. Clearly, transfer of stock to susceptible sites post November allows approximately 10 months growth before potential infestation in the following September. Management strategies will necessarily be site specific and depend on the stocking size, growth rate and marketed size of stock. Placement of stock at approximately 25 mm in November may allow growth, under ideal conditions, to approximately 55-60 mm during the next year. This represents the probable minimum market size for abalone and stock grown under this regime would, thus, be subjected to little *B. knoxi* influence. Where there is a requirement to grow stock to larger sizes e.g. 80-100 mm, potential exposure to *B. knoxi* settlement in the second year may necessitate a treatment regime as discussed below.

Larval settlement data for *P. hoplura* (Chapter 3) suggested that initial colonization with this species could substantially be avoided by placement of stock post spring and preferably after mid summer. Once established in abalone, however, the species showed the potential for year round lecithotrophic reproduction. Thus numbers of this species have the potential to rise steadily over time and this needs to be taken into account and monitored by growers – especially if the grow out period extends over 2-3 years in the sea.

A second component of the spionid management strategy is that of treatment by air drying. Air drying of stock has a history of use in oyster culture (Smith 1984, Nell and Smith 1988) and is the basis of the success of intertidal oyster culture which sacrifices feeding opportunities (when immersed) for the treatment benefits when exposed. Spionid treatment data (Chapter 5) showed that abalone consistently survived 2-4 h air drying, sufficient to significantly reduce mud worm levels. Exposure data further revealed that blacklip abalone could survive 11-15 h out of water under conditions appropriate for elimination of spionids. There may be differential drying tolerances between haliotid species requiring investigation in the future. Short term treatment air drying may potentially, but not necessarily, reduce the growth rate of stock. To minimize stock stress it is suggested that the differential between air and sea temperature be minimized, temperatures > 24 °C should be avoided and stock should be starved for several days before treatment in line with the findings of Watanabe et al. (1994)(Chapter 5.2.4).

As infestation with approximately 5 spionids may reduce growth by about 25% (Chapter 6.2 and Chapter 7) as may, potentially, air drying treatment (Chapter 5.2), infestation at this level or above is considered worth treating. In practice this decision will be based on the duration of time remaining before stock can be sold and on the spionid species involved. For instance, it may be counterproductive to treat recent infestations if stock can be sold within a few months. Growers should also consider that *B. knoxi* numbers will not increase in stock until the following spring dispersal period whereas *P. hoplura* counts have the potential to rise steadily from a low level of infestation to >50 per abalone in a 2 year period (Chapter 7). It is recommended that abalone infested with greater than 10 - 20 spionids early in the grow-out phase are treated by air drying, as on balance, the risks associated with no treatment appear greater than the potential consequences of treatment side-effects. Whether abalone can be appropriately air dried *in situ* within rearing vessels will depend largely on the degree of fouling acquired by such vessels and their degree of enclosure. The Aquatek© trays (Chapter 2) with lids removed allow appropriate drying of stock as compared to more enclosed designs such as tubes (Chapter 2). Abalone culturists may find that air drying treatment is best combined with other

handling activity such as grading or thinning out of rearing vessels. In such situations sheet plastic may serve as an appropriate substrate during drying.

In addition to avoidance strategies based on spionid reproduction and settlement, the research identified other factors of importance in minimizing spionid impacts. Tube building polychaetes, especially *Spirorbis* sp., when present on abalone were found to enhance settlement of *B. knoxi*. Culture facilities in susceptible areas are advised to source spat without significant quantities of these fouling organisms. Fortunately, in southern Tasmania the settlement season for spirorbids is similar to that of spionids (Chapter 6.2.3). Therefore, stocking regimes designed to avoid mud worm infestation in the first year will result in minimal spirorbid fouling and, thus, relief from the *B. knoxi* enhancement effect for a substantial time (Chapter 6.2). Control of spirorbids in land-based farms may require further research. When stock of several size classes were exposed to spionids at the same time, larger stock became significantly more infested. By contrast, as small minimally affected abalone grew from approximately 20 to 60 mm over 2 years, spionid infestation remained at very low levels. Abalone reared closer to the bottom were found to recruit higher numbers of *P. hoplura*. Thus, it may be prudent to raise culture vessels from the bottom as has been recommended previously for oyster culture (Chapter 6. 4). Review of general principles regarding spionid abundance suggest that suitable sites for abalone culture should exclude very sheltered, static sites with muddy sediments near estuaries and sources of organic pollution.

As discussed above, where experimentally exposed abalone became heavily spionid infested, mortality could only be attributed to the combination of severe shell blistering and underfeeding but not to significant infestation as a sole known stress factor. Spionid infestation led to reduced growth and a decrease in percentage flesh weight. These gross changes were manifestations of disruption to normal physiology and biochemistry, including an increase in oxygen consumption and utilization and reduction of tissue glycogen and protein stores. These latter observations were reflected in morphological changes in the right kidney and digestive gland.

Thus, the health of abalone was compromised, possibly because resources were diverted from normal functions to repair of the shell. Severely spionid compromised abalone also showed a reduced capacity to regulate potassium. Long-term monitoring of potassium haemolymph levels in stocks before and during the summer could be a useful addition to health studies on so called "summer stress syndrome" which has emerged as a serious problem for land-based abalone farms in parts of Australia and is linked to elevated water temperature and bacterial infection.

The general principle of minimizing spionid impact by reduction of risk factors, including timing of settlement and treatment by air drying, if necessary, is applicable to abalone farming using other culture technology and in different locations. As abalone farming expands into new locations and production increases it is likely that severe spionid infestations will be encountered from time to time. Vigilance will be required!

The following is a list of the principal writers who have written on the worm and its habits :—

Leucodore ciliatus—Johnston, Magazine of Zoology and Botany, 1838, ii., p. 66, pl. 3, f. 1-6.

" " Dr. T. Williams, Report of the British Association, 1851, p. 208.

" " Dr. Johnston, Catalogue of Non-Parasitical Worms in the British Museum, 1865, p. 205, pl. 18, f. 6.

" " Prof. E. Ray Lankester, Annals and Magazine of Natural History, 1868, vol. 1, ser. 4, p. 233, pl. xi.

" " Prof. W. C. McIntosh, Annals and Magazine of Natural History, vol. 2, ser. 4, 1868, p. 276, pls. xviii. and xix.

Leucodore ciliatus—Prof. T. H. Huxley, The English Illustrated Magazine, No. 1, Oct. 1883, pp. 46 to 52; No. 2, Nov. 1883, pp. 112 to 121.

" " Dr. W. A. Haswell, Centennial Magazine, Sept. 1889, p. 148.

Polydora (Leucodore) ciliata (Johnston)—Alexr. Agassiz, Annals and Magazine of Natural History, vol. xix, ser. 3, 1867, p, 242, pls. v. and vi.

Polydora (Leucodore) ciliata—Dr. W. A. Haswell, Proceedings of the Linnæan Society of New South Wales, vol. x, p. 273.

There are very many other papers bearing on the habits of the worm, amongst which may be mentioned one by Dr. Wright in the Edinburgh New Philosophical Journal, 1857, vol vi, p. 90; another by Mr. Alexander Oliver in the Centennial Magazine for September, 1889, pp. 134 to 148; and some details of the habits of the worm are given by Sir J. Dalyell in his work on the Powers of the Creator Displayed in Creation, 1851, vol. ii, p. 159.

--From the reference section of the report by Thomas Whitelegge (1890): "Report on the worm disease affecting the oysters on the coast of New South Wales".

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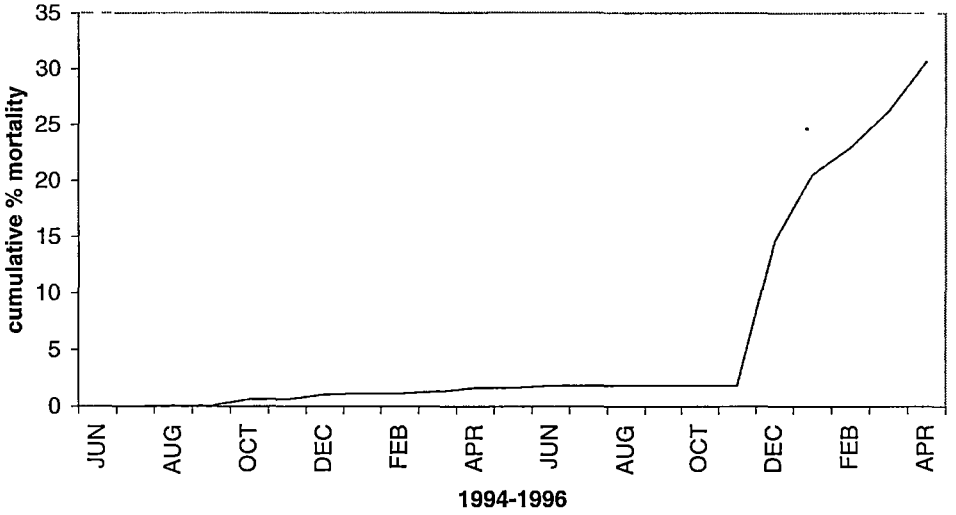
Appendices

Appendix 1A Mortality data for Huon Aquaculture Company

	Initial	Intake	MORTALITY DATA						
Species	No.	Date	1995 DEC	1996 JAN	1996 FEB	1996 MAR	1996 APR	TOTALS	%kill
RUBRA	220	14.06.94	74	13	2	7	13	109	50
RUBRA	220	14.06.94	48	25	8	13	6	100	45
RUBRA	220	14.06.94	12	10	0	5	4	31	14
LAEVIGATA	129	22.06.94	11	7	4	2	12	36	28
RUBRA	220	14.06.94	36	7	4	6	7	60	27
RUBRA	220	14.06.94	13	10	9	16	6	54	25
LAEVIGATA	129	22.06.94	24	19	3	3	7	56	43
RUBRA	220	14.06.94	7	5	7	6	9	34	15
RUBRA	220	14.06.94	5	15	5	1	9	35	16
RUBRA	220	14.06.94	28	8	7	7	17	67	30
Totals:	2018		258	119	49	66	90	582	29%

	Initial	Intake	MORTALITY DATA						
Species	No.	Date	1995 DEC	1996 JAN	1996 FEB	1996 MAR	1996 APR	TOTALS	%kill
H .laevigata	120	14.08.95	15	6	4	4	3	32	26.7
H .laevigata	120	14.08.95	24	14	4	10	6	58	48.3
H .laevigata	120	14.08.95	31	5	4	5	1	46	38.3
Hybrid	60	14.08.95	6	0	0	5	0	11	18.3
Hybrid	60	14.08.95	3	0	3	3	1	10	16.7
Hybrid	60	14.08.95	5	1	4	3	2	15	25
Totals:	540		84	26	19	30	13	172	32%

	Initial	Intake	MORTALITY DATA						
Species	No.	Date	1995 DEC	1996 JAN	1996 FEB	1996 MAR	1996 APR	TOTALS	%kill
H. rubra	200	05.01.95	2	0	67	3	0	72	7
H. rubra	200	05.01.95	0	1	0	11	12	24	36
H. rubra	200	05.01.95	0	0	6	2	1	9	12
H. rubra	100	05.01.95	0	0	0	5	0	5	4.5
H. rubra	100	05.01.95	0	0	6	2	1	9	5
H. rubra	150	05.01.95	1	0	4	4	4	13	9
H. rubra	150	05.01.95	0	0	0	0	2	2	8.7
H. rubra	200	05.01.95	2	2	4	0	2	10	1.3
H. rubra	200	05.01.95	2	6	11	4	3	26	5
H. rubra	200	05.01.95	0	0	2	2	7	11	13
H. rubra	200	05.01.95	0	0	9	2	3	14	5.5
H. rubra	200	05.01.95	2	0	25	15	10	52	26
H. rubra	200	05.01.95	4	9	6	10	1	30	15
H. rubra	200	05.01.95	3	3	3	12	10	31	15.5
	2500		16	21	143	72	56	308	12.3%



Appendix 1A Figure 1. Cumulative mortality for June 1994 abalone intake at Huon Aquaculture Company.

Appendix 1B Subjective shell damage ratings (SSDR) for remnant Huon Aquaculture Company abalone sampled 1997-1998.

SSDR	0	1	2	3
% each grade	1	15	52	36

n = 149, shell kept and assessed retrospectively May 2001

Appendix 1C Annual Pacific Oyster blister frequency data

Year	% stock with shell blisters	No. oysters
1999	6.7	1600
1998	8.6	1890
1996	1.9	1250
1995	10.2	680
1994	6.8	1320
1990-92	4.0*	>5000

* Data from Wilson et al. (1993)
Other data from DPIWE Fish Health Lab records

Appendix 1D. SSDR scores for wild abalone from Tasmania (% values in parentheses)

SSDR score	0	1	2	3
Greenlip	45(64.3)	11(15.7)	9(12.9)	5(7.1)
Blacklip	101(24.0)	166(39.5)	143(34.0)	10(2.4)

n=490, some shell damage may be due to boring sponge
Chi-square analysis found significant difference between species (P<0.001)

Appendix 1E Comparison of SSDR scores between stunted and normal population samples (% values in parentheses)

SSDR score	0	1	2	3
Normal	143(31.1)	166(36.1)	137(29.8)	14(3.0)
"stunted"	3 (10)	11(36.7)	15(50)	1(3.3)

Chi-square analysis found significant difference between size groups (P<0.05)

Appendix 4 Immersion treatment screening data

4A fresh water

Spionids *in vitro*	Time (min)			
survival	10	15	30	45
48 h post treatment	0/5	0/5	0/5	0/5

Temperature 18 °C

Spionids *in situ*, $\overline{X}$ (SD)	Fresh water Immersion Time (min.)			
	control	30	60	120
mean chimneys	21.6(8.1)	30.4(14.8)	26.2(11.8)	18.3(8.3)
mean surviving *B. knoxi*	26.5(10.1)	32.8(13.7)	30.6(15.2)	16.0(13.5)
mean *B. knoxi* (EI)% Kill	0.3(0.9)	8.6(14.7)	13.7(26.4)	30.2(33.3)
(GMC)% Kill –*B. knoxi*	-	0	0	39.6
mean survival – Total worms	27.0(10.0)	32.9(13.7)	31.1(15.4)	16.3(13.6)
(GMC)%Kill – Total worms	-	0	0	39.6
% Abalone mortality	0	10	50	80

n=10 all treatments (5/10 live in control group)

4B potassium permanganate

Spionids *in vitro*	Concentration KMnO4 ($mg.l^{-1}$)					
Survival	2	4	8	15	20	50
7 d post treatment	1/5	5/5	4/5	3/5	2/5	0/5

Temperature 18 °C

Spionids *in situ*, $\overline{X}$ (SD)	Concentration KMnO4 ($mg.l^{-1}$)	
	control	25
mean surviving *B. knoxi*	15.9(11.3)	20.6(26.9)
(GMC)% Kill –*B. knoxi*		0
mean survival – Total worms	33.2(23.9)	29.5(40.6)
(GMC)%Kill – Total worms		11.2
% Abalone mortality	0	60

n=19 each group

$KMnO_4$ toxicity to abalone. Combined data for 3 exposure time and concentration experiments.

40-50 mm abalone	5 $mg.l^{-1}$	10 $mg.l^{-1}$	15 $mg.l^{-1}$	20 $mg.l^{-1}$	25 $mg.l^{-1}$	30 $mg.l^{-1}$	50 $mg.l^{-1}$
Mortality 14 d post treatment (3 h)			0/5		0/5		1/5
Mortality 16 d post treatment (4 h)		0/5		4/5		4/5	
Mortality 8 d post treatment (9 h)	0/10						

4C gentian violet

Spionids *in vitro*	Concentration of Gentian Violet ($mg.l^{-1}$)				
Survival	control	10	20	50	100
Immediate post treatment	10/10	3/5	3/5	2/5	0/5
4 d post treatment	9/10	0/5	0/5	0/5	0/5

Temperature 15°C

Appendix 4C continued.....

Spionids *in situ*, $\overline{X}$ (SD)	Concentration gentian violet ($mg.l^{-1}$)	
	control	7.5
mean surviving *B. knoxi*	11.5(7.1)	10.0(7.8)
(GMC)% Kill -*B. knoxi*		13.0
mean survival – Total worms	18.4(10.6)	14.1(9.0)
(GMC)%Kill -Total worms		23.4

n=18 each group

Gentian violet toxicity to abalone. Data for 2 exposure time and concentration experiments

40-50 mm abalone	3 $mg.l^{-1}$	5 $mg.l^{-1}$	10 $mg.l^{-1}$	20 $mg.l^{-1}$
Mortality after 17 d (3 h exposure)		0/5	2/5	3/5
Mortality after 11 d (9 h exposure)	1/10			

4D mebendazole

Spionids *in situ*	Concentration of mebendazole ($mg.l^{-1}$)				
Survival	control	50	100	200	500
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
9 days post treatment	5/5	0/5	0/5	1/5	0/5

Temperature 16 °C

Spionids *in situ*, $\overline{X}$ (SD)	Concentration of Mebendazole ($mg.l^{-1}$)	
	control	200
mean surviving *B. knoxi*	4.9(4.1)	2.0(1.7)
(GMC)% Kill -*B. knoxi*		59.2
mean survival – Total worms	8.8(9.6)	4.1(3.8)
(GMC)%Kill -Total worms		53.4

n=20 each group

Mebendazole toxicity to abalone. Combined data one week post exposure for three exposure time and concentration experiments

40-50 mm abalone	25 $mg.l^{-1}$	50 $mg.l^{-1}$	200 $mg.l^{-1}$
3 hour exposure		0/5	0/5
9 hour exposure		0/10	
17 hour exposure	0/10		

4E fenbendazole

Spionids *in vitro*	Concentration of fenbendazole ($mg.l^{-1}$)				
Survival	control	50	100	200	500
Immediate post treatment	10/10	5/5	5/5	5/5	4/5
10 Days post treatment	9/10	0/5	0/5	1/5	0/5

Temperature 15 °C

Spionids *in situ*, $\overline{X}$ (SD)	Concentration of fenbendazole ($mg.l^{-1}$)	
	Control	250
mean surviving *B. knoxi*	10.9(6.5)	7.6(7.6)
(GMC)% Kill -*B. knoxi*		30.3
mean survival – Total worms	14.0(6.3)	8.3(7.7)
(GMC)%Kill -Total worms		40.7

n=20 each group

Appendix 4E continued.....

fenbendazole toxicity to abalone (3 h exposure)

40-50 mm abalone	100 mg.l-1	250 mg.l-1
Mortality 19 d post treatment	0/4	0/6

Appendix 4F levamisole

Spionids *in vitro*. Combined trial data.	Concentration levamisole ($mg.l^{-1}$)					
survival	Control	0.32	3.2	32	128	320
Trial 1 Immediate post treatment	6/6	6/6	6/6	6/6		6/6
8 d post treatment	6/6	4/6	1/6	5/6		4/6
Trial 2 Immediate post treatment	10/10		5/5	4/5	4/5	4/5
8 d post treatment	9/10		4/5	0/5	1/5	3/5

Temperature 15 -16°C

Spionids *in situ*. $\overline{X}$ (SD)	Concentration of levamisole ($mg.l^{-1}$)				
	Control	6.4	64	640	2 h dry & 64 $mg.l^{-1}$
mean chimneys	42.6(23.9)	40.1(13.5)	30.6(14.1)	38.0(16.9)	41.0(12.9)
mean surviving *B. knoxi*	28.0(14.7)	19.3(9.5)	16.1(6.1)	8.1(6.0)	22.8(8.42)
(GMC)% Kill -*B. knoxi*		31.1	42.5	71.1	18.6
mean survival - Total worms	30.6(14.5)	27.9(19.8)	17.9(5.6)	10.5(4.4)	25.6(8.2)
(GMC)%Kill - Total worms		8.8	41.5	65.7	16.4
% Abalone mortality	0	16.7	50	100	60

n=10 infested shells, n=5-6 abalone mortality data

levamisole exposure data at 320 $mg.l^{-1}$, mudworms *in situ*, $\overline{X}$ (SD)		
	control	levamisole
mean surviving *B. knoxi*	4.9(4.1)	3.2(2.5)
(GMC)% Kill -*B. knoxi*		34.7
mean survival - Total worms	8.8(9.6)	4.9(3.4)
(GMC)%Kill –Total worms		44.3

n=20 each group

levamisole toxicity to abalone (exposure 3 h). Combined data for two stock sizes

40-50 mm abalone	0.32 $mg.l^{-1}$	3.2 $mg.l^{-1}$	32.0 $mg.l^{-1}$	320 $mg.l^{-1}$
Mortality 18 d post treatment	0/5	0/5	1/5	0/5

18-20 mm abalone	64 mg.l-1	320 mg.l-1	512 mg.l-1
Mortality 8 d post treatment	0/15	5/15	15/15

4G malachite green

malachite green toxicity to abalone data (exposure time 3 h)

18-20 mm abalone	5 mg.l-1	10 mg.l-1	20 mg.l-1
Mortality 8 days post treatment	1/10	11/12	12/12

Spionids *in situ* and *in vitro*, $\overline{X}$ (SD)	Concentration malachite green ($mg.l^{-1}$)			
	control	1	5	10
Mean surviving *B. knoxi*	35.1(10.9)	28.4(21.5)	20.0(13.8)	17.2(10.8)
(GMC)% Kill -*B. knoxi*		19.1	43.0	51.0
Mean survival – Total worms	58.7(16.6)	51.5(38.3)	36.6(15.0)	23.7(14.6)
(GMC)%Kill – Total worms		12.3	37.6	59.6
Spionid survival immed. post treat.	22/22	12/12	17/17	31/31
Spionid survival 7 post treat.	22/22	12/12	0/17	0/31
n=10 each abalone treatment				

4H trichlorofon

Spionids *in vitro*	Concentration of trichlorofon ($mg.l^{-1}$)						
survival	control	0.1	1	10	100	500	1000
Immediate post treatment	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8 d post treatment	4/5	5/5	5/5	5/5	5/5	4/5	4/5

Temperature 13 °C

Trichlorofon toxicity to abalone (exposure time 3 h)			
40-50 mm abalone	10 $mg.l^{-1}$	100 $mg.l^{-1}$	500 $mg.l^{-1}$
Mortality 11 d post treatment	0/5	0/5	0/5

4I Praziquantel

Spionids *in vitro*	Concentration of Praziquantel ($mg.l^{-1}$)						
survival	control	0.5	1	5	10	50	100
Immediate post treatment	6/6	6/6	6/6	6/6	6/6	6/6	6/6
20 d post treatment	5/6	6/6	6/6	6/6	4/6	6/6	5/6

Temperature 14 °C

4J Hydrogen peroxide

Spionids *in vitro*	Concentration of Hydrogen Peroxide (ppm)					
Survival	control	50	100	200	500	1000
Immediate post treatment	5/5	5/5	5/5	5/5	5/5	5/5
9 d post treatment	4/5	5/5	5/5	3/5	3/5	3/5

Temperature 15 °C

Hydrogen peroxide toxicity to abalone (exposure time 3 h)		
40-50 mm abalone	50 $mg.^{l-1}$	200 $mg.^{l-1}$
Mortality 14 d post treatment	0/5	0/5

4K Formalin

Formalin toxicity to abalone (3h exposure)			
18-20 mm abalone	50 mg.l-1	100 mg.l-1	200 mg.l-1
Mortality after 7 d	0/10	0/10	9/10

Spionids *in vitro* and *in situ*, $\overline{X}$ (SD).	Concentration of Formalin (ppm)			
	control	50	100	200
mean surviving *B. knoxi*	28.7(14.8)	22.9(10.0)	25.5(16.0)	18.3(6.1)
(GMC)% Kill -*B. knoxi*		20.2	11.1	36.2
mean survival - Total worms	39.1(21.9)	37.2(14.6)	30.3(16.5)	19.9(7.2)
(GMC)%Kill - Total worms		4.9	22.5	49.1
spionid survival immed. post treat.	5/5	15/15	10/10	6/6
spionid survival 8 d post treat.	4/5	14/15	7/10	1/6

4L Ivermectin

Spionids *in situ*, $\overline{X}$ (SD).	Concentration Ivermectin ($mg.l^{-1}$)			
	control	0.004	0.04	0.4
mean surviving *B. knoxi*	20.9(11.6)	30.1(18.5)	28.7(18.1)	8.3(3.7)
(GMC)% Kill –*B. knoxi*		0	0	60.3
mean survival – Total worms	25.5(11.2)	37.5(30.6)	32.1(22.2)	14.5(7.3)
(GMC)%Kill – Total worms		0	0	56.9
% Abalone mortality	33.3	23.1	46.2	84.6

n=10 infected abalone, n=13 for mortality data

Spionids *in vitro*	Concentration of Ivermectin ($mg.l^{-1}$)				
Survival	control	0.05	0.1	0.2	0.3
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
7 d post treatment	5/5	5/5	2/5	5/5	5/5

Temperature 16°C

Ivermectin toxicity to abalone data (3 h exposure)

40-50 mm abalone	0.05 $mg.l^{-1}$	0.1 $mg.l^{-1}$	0.2 $mg.l^{-1}$	0.3 $mg.l^{-1}$
Mortality after 18 d	0/4	1/4	4/4	4/4

4M Exposure to febantel, pyrantel embonate and praziquantel in combination.

Spionids *in vitro*	Concentration of Febantel and Pyrantel Embonate ($mg.l^{-1}$) *				
Survival	control	25 & 14.4	50 & 28.8	125 & 72	250 & 144
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
8 d post treatment	0/5	2/5	4/5	4/5	lost

* Febantel concentration shown first in column headings, praziquantel not shown as previously shown ineffective. Temperature 15°C

Abalone mortality data. Exposure to febantel, pyrantel embonateand praziquantel in combination

40-50 mm abalone	250mg/ l & 144 $mg.l^{-1}$ *
Mortality after 15 d	0/5

* Febantel concentration shown first, praziquantel not shown as above.

4N Metronidazole & Dimetronidazole

Spionids *in vitro*	Concentration metronidazole ($mg.l^{-1}$)					
Survival	5	10	20	50	100	200
48 hr post treatment	10/10	10/10	7/10	9/10	7/10	10/10

Temperature 18 °C

Appendix 4N continued.....

Spionids *in vitro*	Concentration dimetronidazole ($mg.l^{-1}$)					
Survival	Control	20	50	100	200	500
Immediate post treatment	5/5	5/5	5/5	5/5	5/5	5/5
8 d post treatment	2/5	0/5	3/5	1/5	3/5	4/5

Temperature 16 °C (but with large fluctuation)

4O methylene blue

Spionids *in situ*, $\overline{X}$ (SD).	Concentration of methylene Blue ($mg.l^{-1}$)			
	control	1	5	10
mean surviving *B. knoxi*	20.6(6.3)	32.7(10.9)	23.0(10.4)	23.1(10.8)
(GMC)% Kill -*B. knoxi*		0	0	0
mean survival – Total worms	30.9(7.2)	39.3(12.7)	30.5(11.8)	35.2(12.1)
(GMC)%Kill – Total worms		0	1.3	0
% Abalone mortality	0	0	0	0

n=10 infected shells, n=3 mortality data

Spionids *in vitro*	Concentration methylene Blue ($mg.l^{-1}$)				
Survival	20	30	50	100	200
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
7 d post treatment	4/5	2/5	4/5	5/5	5/5

Temperature 18 °C

Appendix 5A ANOVA table for clinical pathology indicators from 3.5 h air dried abalone from two bleed sites

Variate: Cu

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	8734	2183	0.28	
Time	2	158279	79140	10.25	<0.001
Site	1	36	36	0.00	0.947
Time.Site	1	134	67	0.01	0.991
Residual	19(1)	146742	7723		
Total	28(1)	308467			

Variate: Cl

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	1024.80	256.2	3.12	
Time	2	7640.27	3820.13	46.51	<0.001
Site	1	229.63	229.63	2.8	0.110
Time.Site	2	91.47	45.73	0.56	0.582
Residual	20	1642.8	82.14		
Total	29	10628.97			

Variate: K

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	2.0487	0.5122	0.58	
Time	2	32.4047	16.2023	18.50	<0.001
Site	1	12.4163	12.4163	14.18	0.001
Time.Site	2	0.0487	0.0243	0.03	0.973
Residual	20	17.5153	0.8758		
Total	29	64.4337			

Variate: Na (2 values with high residuals deleted)

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	311.89	77.97	2.26	
Time	2	10194.54	5097.27	147.75	<0.001
Site	1	264.59	264.59	7.67	0.013
Time.Site	2	86.36	43.18	1.25	0.310
Residual	18(2)	620.98	34.5		
Total	27(2)	11087.25			

Variate:Na/K ratio (4 values with extreme residuals deleted)

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	17.107	4.277	3.17	
Time	2	125.974	62.987	46.66	<0.001
Site	1	121.086	121.086	89.69	<0.001
Time.Site	2	0.577	0.289	0.21	0.810
Residual	16(4)	21.6	1.35		
Total	25(4)	267.885			

Variate: Ca

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	0.9836	0.2459	2.03	
Time	2	5.6221	2.8111	23.20	<0.001
Site	1	0.3081	0.3081	2.54	0.127
Time.Site	2	0.0405	0.0203	0.17	0.847
Residual	20	2.4236	0.1212		
Total	29	9.3779			

Variate: Mg

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	14.984	3.746	1.62	
Time	2	157.793	78.897	34.12	<0.001
Site	1	8.269	8.269	3.58	0.073
Time.Site	2	1.452	0.726	0.31	0.734
Residual	20	46.251	2.313		
Total	29	228.749			

Variate: Glucose

Source	df	SS	MS	VR	F prob.
Rep stratum	4	0.04533	0.01133	0.92	
Time	2	0.09267	0.04633	3.76	0.041
Site	1	0.01200	0.01200	0.97	0.336
Time.Site	2	0.00200	0.00100	0.08	0.922
Residual	20	0.24667	0.01233		
Total	29				

Variate: Protein

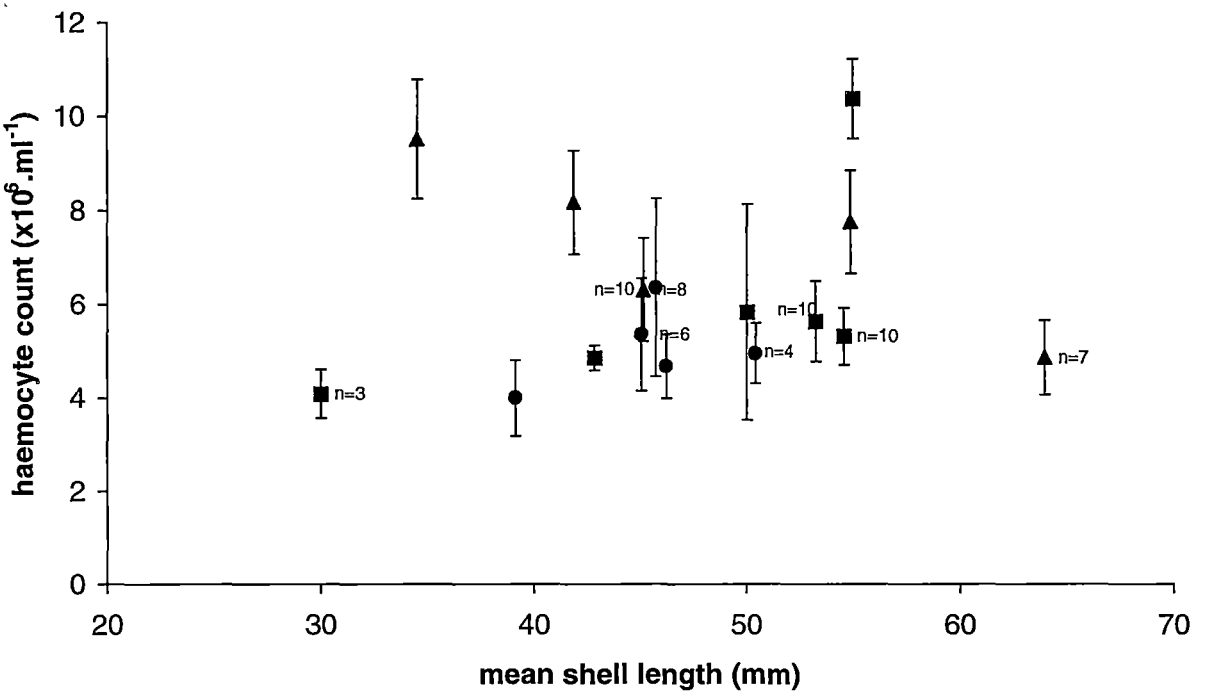
Source	Df	SS	MS	VR	F prob.
Rep stratum	4	10.960	2.740	0.57	
Time	2	112.233	56.116	11.64	<0.001
Site	1	0.616	0.616	0.13	0.724
Time.Site	2	2.129	1.064	0.22	0.804
Residual	20	96.424	4.821		
Total	29	222.362			

Variate: Haemocyte count (cephalic sinus only)

Source	Df	SS	MS	VR	F prob.
Rep stratum	4	9406	2351	1.25	
Time	2	16526	8263	4.39	0.052
Site	8	15064	1883		
Total	14	40996			

Appendix 5B

Haemocyte count data by foot incision method for 16 populations of presumptive healthy abalone (means ± SE, n=5 unless otherwise indicated). ■ = Farm 1 samples, ● = Tasmanian Scallops samples, ▲ = Huon Aquaculture samples. Mean haemocyte count is 6.1×10^6 cells.ml^{-1} (SD=1.9×10^6 n=16 populations)



Appendix 5C ANOVA table for haemolymph pH data for 5 h air dried abalone from two bleed sites

Source	Df	SS	MS	VR	F prob.
Site	1	0.023805	0.023805	3.05	0.086
Time	6	0.924608	0.154101	19.71	<0.001
Site.Time	6	0.132059	0.022010	2.82	0.017
Residual	66	0.515899	0.007817		
Total	79	1.596371			

Appendix 6A

Descriptive Statistics:
Aquatas Data. Mud worm settlement on three size classes of abalone.
means with standard error in parenthesis.

	Small	Medium	Large
B. knoxi chimneys	0.0 (0.0)	0.5 (0.3)	8.6 (0.7)
B. knoxi	0.0 (0.0)	0.4 (0.1)	4.2 (0.8)
P. hoplura	0.9 (0.2)	1.7 (0.5)	12.8 (2.4)

Displayed means are replicates of 5 abalone, n=14, 17 and 21 for small, medium and large size classes respectively.

Comparison Statistics: replicates pooled

Kruskal-Wallis test, Aquatas **Chimney count** data

$H_{calculated} = 41.640 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	23.22353	4.56398	5.088438	2.394	reject Ho
Big V Small	27.9	4.821062	5.787106	2.394	reject Ho
Small V Med	4.676471	4.993151	0.936577	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Kruskal-Wallis test, Aquatas ***B. knoxi*** **count** data

$H_{calculated} = 26.343 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	16.49118	4.56398	3.613332	2.394	reject Ho
Big V Small	22.05	4.821062	4.573681	2.394	reject Ho
Small V Med	5.558824	4.993151	1.11329	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Kruskal-Wallis test, Aquatas ***P. hoplura*** **count** data

$H_{calculated} = 34.082 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean separation

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	23.11912	4.56398	5.065561	2.394	reject Ho
Big V Small	25.93214	4.821062	5.378927	2.394	reject Ho
Small V Med	2.813025	4.993151	0.563377	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Appendix 6B

Descriptive Statistics:
Huon Aquaculture Data. Mud worm settlement on three size classes of abalone.
Means with standard error and n in parentheses.

	Replicate	Small	Medium	Large
B. knoxi chimneys	1	0.0 (0.0, 3)	0.8 (0.7, 6)	7.9 (1.0, 9)
B. knoxi		0.3 (0.6, 3)	0.8 (0.7, 6)	5.1 (0.5, 9)
P. hoplura		1.7 (0.7, 3)	5.8 (1.1, 6)	39.0 (6.4, 9)
B. knoxi chimneys	2	-	1.9 (0.4, 7)	12.2 (1.4, 10)
B. knoxi		-	0.9 (0.5, 7)	4.4 (0.6, 10)
P. hoplura		-	1.0 (0.2, 7)	21.3 (7.1, 10)

Comparison Statistics - by replicate

Replicate 1
Kruskal-Wallis test: Huon Aquaculture Company **Chimney count** data

$H_{calculated} = 13.279 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	8.222222	2.714642	3.028842	2.394	reject Ho
Big V Small	9.888889	3.433781	2.879884	2.394	reject Ho
Small V Med	1.666667	3.642074	0.457615	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Replicate 1
Kruskal-Wallis test: Huon Aquaculture Company ***B. knoxi*** **count** data

$H_{calculated} = 12.132 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	8.361111	2.714642	3.080005	2.394	reject Ho
Big V Small	8.944444	3.433781	2.604839	2.394	reject Ho
Small V Med	0.583333	3.642074	0.160165	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Replicate 1
Kruskal-Wallis test: Huon Aquaculture Company ***P. hoplura*** **count** data

$H_{calculated} = 14.101 > H_{critical}$ ($X^2_{0.05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	7.583333	2.714642	2.793493	2.394	reject Ho
Big V Small	11.83333	3.433781	3.446153	2.394	reject Ho
Small V Med	4.25	3.642074	1.166917	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Replicate 2
Mann-Whitney U test: Huon Aquaculture Company (small size class lost).

Medium Vs Large size class. Mann-Whitney U Test

	B. knoxi chimneys	*B. knoxi*	*P. hoplura*
Value of U	2.5	4.5	0.00
Normal approximation	3.201	3.010	3.416
P value	<0.01	<0.01	<0.01

normal approximation adjusted for ties

Appendix 6C (section 6.1 three size experiment: shell damage and fouling data)

% blister data for 3 size classes at Aquatas (combined replicates)

$H_{calculated} = 32.150 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	25.825	5.522681	4.676171	2.394	reject Ho
Big V Small	28.25	5.522681	5.11527	2.394	reject Ho
Small V Med	2.425	5.522681	0.439098	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

% blister data for 3 size classes at Huon Aquaculture Company (combined replicates)

$H_{calculated} = 35.095 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	24.3	4.682097	5.189982	2.394	reject Ho
Big V Small	28.53	6.193832	4.606195	2.394	reject Ho
Small V Med	4.23	6.193832	0.682937	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

SSDR for 3 size classes at Aquatas (combined replicates)

$H_{calculated} = 28.217 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	22.95	5.522681	4.155591	2.394	reject Ho
Big V Small	27.3	5.522681	4.943252	2.394	reject Ho
Small V Med	4.35	5.522681	0.787661	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

SSDR for 3 size classes at Huon Aquaculture Company (combined replicates)

$H_{calculated} = 54.007 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	50.77863	8.152578	6.228537	2.394	reject Ho
Big V Small	69.93889	12.93187	5.408257	2.394	reject Ho
Small V Med	19.16026	13.33992	1.43631	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Aquatas: fouling data (combined replicates)

$H_{calculated} = 34.068 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	18.1	5.522681	3.277394	2.394	reject Ho
Big V Small	32.15	5.522681	5.821448	2.394	reject Ho
Small V Med	14.05	5.522681	2.544054	2.394	reject Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Huon Aquaculture Company: fouling data (combined replicates)

$H_{calculated} = 32.858 > H_{critical}$ ($X^2_{0\,05,\,2} = 5.991$, from Table B1. Zar 1984)
Mean Separation:

comparison	Δ mean ranks	SE	Q	$Q_{0\,05,3}$	conclusion
Big V Med	24.32	4.939636	4.92344	2.394	reject Ho
Big V Small	29.92	6.534524	4.578758	2.394	reject Ho
Small V Med	5.6	6.534524	0.856987	2.394	Accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Appendix D (section 6.2 – spionids and fouling)

Descriptive Statistics

Aquatas Site.
Mud worm settlement indicators and SSDR
for "clean" and spirorbid fouled stock. Means with SE and n in parentheses.

Fouling status	*B. knoxi* chimneys	*B. knoxi*	*P. hoplura*	SSDR
"clean"	0.3 (0.1, 70)	0.9 (0.2,14)	0.1 (0.1, 14)	0.1 (0.1,70)
"fouled"	3.2 (0.5, 20)	9.0 (1.6, 4)	0.0 (0.0, 4)	1.1 (0.1,20)

Huon Aquaculture Site.
Replicate 1. Mud worm settlement indicators and SSDR
for "clean" and spirorbid fouled stock. Means with SE and n in parentheses

Fouling status	*B. knoxi* chimneys	*B. knoxi*	*P. hoplura*	SSDR
"clean"	0.3 (0.1, 50)	1.2(0.6, 10)	0.1 (0.1,10)	0.2 (0.1,50)
"fouled"	3.1 (0.5, 20)	9.5 (1.6,4)	2.3 (1.0, 4)	1.3 (0.1, 20)

Huon Aquaculture Site.
Replicate 2. Mud worm settlement indicators and SSDR
for "clean" and spirorbid fouled stock. Means with SE and n in parentheses

Fouling status	*B. knoxi* chimneys	*B. knoxi*	*P. hoplura*	SSDR
"clean"	0.8 (0.2,50)	3.0 (0.5, 10)	1.1 (0.6, 10)	0.6 (0.1,50)
"fouled"	4.9 (0.9, 12)	19.0 (3.0, 2)	4.5 (3.5, 2)	1.1 (0.2, 12)

Comparison Statistics

Aquatas:
Spirorbid fouled Vs clean stock for *B. knoxi* chimney counts and SSDR. Mann-Whitney U Test

	Chimney counts	SSDR
Value of U	83.0	125.5
Normal approximation	7.078	6.907
p value	0.00	0.00

normal approximation adjusted for ties

Aquatas: Spirorbid fouled Vs clean stock for *B. knoxi* and *P. hoplura* worms.
Kolmogorov - Smirnov Two -Sample Test

	B. knoxi	*P. hoplura*
Maximum difference	1.0000	0.07
Chi-squared value (2 df)	12.44	0.0634
P	0.00	0.97

Huon Aquaculture: Replicate 1
Spirorbid fouled Vs clean stock for *B. knoxi* and *P. hoplura* worms.
Kolmogorov - Smirnov Two -Sample Test

	B. knoxi	*P. hoplura*
Maximum difference	0.9000	0.9000
Chi-squared value (2 df)	9.257	9.257
P	0.01	0.01

Huon Aquaculture: Replicate 1
Spirorbid fouled Vs clean stock for *B. knoxi* chimney counts and SSDR. Mann-Whitney U Test

	Chimney counts	SSDR
Value of U	88.0	81.5
Normal approximation	6.260	6.246
p value	0.00	0.00

normal approximation adjusted for ties

Huon Aquaculture: Replicate 2
Spirorbid fouled Vs clean stock for *B. knoxi* and *P. hoplura* worms.
Kolmogorov-Smirnov Two -Sample Test

	B. knoxi	*P. hoplura*
Maximum difference	1.000	0.600
Chi-squared value (2 df)	6.667	2.400
P	0.04	0.30

Huon Aquaculture: Replicate 2

Spirorbid fouled Vs clean stock for *B. knoxi* chimney counts and SSDR. Mann-Whitney U Test

	Chimney counts	SSDR
Value of U	31.5	186.0
Normal approximation	5.022	2.284
p value	0.00	0.02

normal approximation adjusted for ties

Appendix 6E (Section 6.4- Position in the water column)

Position in water column experiment: 6 m Vs 9 m, Mann-Whitney U Test

	B. knoxi chimneys	*B. knoxi*	*P. hoplura*	Total spionids	SSDR
Value of U	179.5	190.5	117.0	124.0	129.0
Normal approximation	0.7955	0.4475	2.652	2.375	2.148
p value	0.43	0.65	0.01	0.02	0.03

normal approximation adjusted for ties

Appendix 7

A. REML Analysis of total % blister data August 1998 Huon Aquaculture cohort 1 (long term treatment experiment)

Fixed term	df	Wald statistic	Pr.
Time	5	60.4	<0.001
Treatment	1	45.4	<0.001
Time.treatment	5	16.1	0.007

B. 2 Way ANOVA for log transformed Boccardia knoxi data
Variate: Log *B. knoxi*

Source of variation	df	ss	Ms	Vr	F pr.
Time	5	3.84530	0.76906	14.33	<0.001
Treatment	1	11.37726	11.37726	212.07	<0.001
Time.treatment	5	1.39603	0.27921	5.20	<0.001
Residual	141	7.56457	0.05365		
Total	152	24.18315			

C. 2 Way ANOVA for log transformed *P. hoplura* data
Variate: *P. hoplura*

Source of variation	df	Ss	Ms	Vr	F pr.
Time	5	35.82420	7.16484	85.02	<0.001
Treatment	1	4.57053	4.57053	54.23	<0.001
Time.treatment	5	2.04699	0.40940	4.86	<0.001
Residual	131	11.03977	0.08427		
Total	142	51.68964			

D. Regression analysis Total blister Vs shell length

	df	ss	ms	vr	F pr.
Regression	1	1033	1033.03	35.28	<0.001
Residual	47	1376	29.28		
Total	48	2409	50.19		

Estimates of parameters

	estimate	se	t(47)	t pr.
Constant	80.26	2.37	33.80	<0.001
Total blisters	-0.3793	0.0639	-5.94	<0.001

Significance of r value: $r_{0.05, 47} = 0.288$, $r_{critical}$ 0.288 < 0.648 ($r_{observed}$), so is significant.

E. Regression analysis Total blister Vs whole weight

	df	ss	Ms	vr	F pr.
Regression	1	2744	2744.1	23.94	<0.001
Residual	47	5387	114.6		
Total	48	8131	169.4		

Estimates of parameters

	estimate	se	t(47)	t pr.
Constant	68.40	4.70	14.56	<0.001
Total blisters	-0.618	0.126	-4.89	<0.001

Significance of r value: $r_{0\,05, 47} = 0.288$, $r_{critical}$ 0.288 < 0.566 ($r_{observed}$), so is significant

F. Regression analysis: Condition Index (LENGTH) Vs weight

	Df	Ss	Ms	vr	F pr.
Regression	1	121.6	121.6	477.09	<0.001
Residual	41	10.5	0.254		
Total	42	132.1	3.14		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	0.742	0.141	5.25	<0.001
Weight	0.20277	0.00928	21.84	<0.001

G. Regression analysis : Condition Index (LENGTH) Vs length

	df	Ss	Ms	vr	F pr.
Regression	1	120.1	120.1	410.67	<0.001
Residual	41	12.0	0.292		
Total	42	132.1	3.14		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	-4.271	0.384	-11.12	<0.001
Length	0.17781	0.00877	20.26	<0.001

H. Regression analysis: % Flesh weight Vs length

	df	Ss	Ms	Vr	F pr.
Regression	1	14.6	14.6	0.72	0.401
Residual	41	830.1	20.3		
Total	42	844.6	20.1		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	66.67	3.20	20.86	<0.001
Length	0.0620	0.0730	0.85	0.401

I. Regression analysis: % flesh weight Vs weight

	df	Ss	ms	Vr	F pr.
Regression	1	17.2	17.2	0.85	0.361
Residual	41	827.4	20.18		
Total	42	844.6	20.11		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	68.35	1.26	54.33	<0.001
Weight	0.0763	0.0826	0.92	0.361

J. Regression analysis: Condition index (weight) Vs length

	df	Ss	ms	vr	F pr.
Regression	1	3.09	3.0875	3.40	0.073
Residual	41	37.26	0.9089		
Total	42	40.35	0.9607		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	3.273	0.677	4.83	<0.001
Length	0.0285	0.0155	1.84	0.073

K. Regression analysis : Condition index (weight) Vs weight

	df	Ss	ms	vr	F pr.
Regression	1	2.50	2.5043	2.71	0.107
Residual	41	37.85	0.9231		
Total	42	40.35	0.9607		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	4.121	0.269	15.32	<0.001
Weight	0.0291	0.0177	1.65	0.107

L. Regression analysis: % DRY WEIGHT Vs length

	df	Ss	ms	vr	F pr.
Regression	1	51.7	51.69	8.42	0.006
Residual	41	251.7	6.14		
Total	42	303.4	7.22		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	12.25	1.76	6.96	<0.001
Length	0.1167	0.0402	2.90	0.006

M. Regression analysis: DRY WEIGHT Vs weight

	df	Ss	Ms	vr	F pr.
Regression	1	30.2	30.198	4.53	0.039
Residual	41	273.2	6.66		
Total	42	303.4	7.22		

Estimates of parameters

	Estimate	se	t(41)	t pr.
Constant	15.943	0.723	22.06	<0.001
Weight	0.1010	0.0475	2.13	0.039

N. 2 Way ANOVA: % flesh weight data August 1998 Huon Aquaculture cohort 1 (long term treatment experiment) Data arcsine transformed

Source of variation	df	Ss	Ms	VR	F pr.
Time	4	0.332745	0.083186	38.92	<0.001
Treatment	1	0.015884	0.015884	7.43	0.007
Time.treatment	4	0.034430	0.008608	4.03	0.004
Residual	122	0.260766	0.002137		
Total	131	0.643825			

Appendix 7O Distribution of spionids in abalone species world-wide

Spionid species	Haliotid species	Stock status	Location	Reference
P. armata	*H. rubra*	cultured	Tasmania	Lleonart 2001 (present study)
	H. rubra	wild	Victoria	Blake and Kudenov 1978
	H. diversicolor	wild	Japan	Kojima and Imajima 1982
	H. tuberculata	wild	Channel Is. ,Britain	Clavier 1989
	H. roei	wild	West Australia	Tas. DPIWE survey*
	H. roei	wild	South Australia	Blake and Kudenov 1978
	H. iris	wild	New Zealand	Rainer 1973, Read 1975
P. caeca	*H. tuberculata*	wild	Channel Is. ,Britain	Clavier 1989
P. ciliata	*H. tuberculata*	wild	Channel Is. ,Britain	Clavier 1989
	H. diversicolor	wild	Japan	Kojima and Imajima 1982
P. flava	*H. tuberculata*	wild	Channel Is. ,Britain	Clavier 1989
	H. diversicolor	wild	Japan	Kojima and Imajima 1982
P. giardi	*H. rubra*	wild	Victoria, Aust.	Blake and Kudenov 1978
P. hoplura	*H. rubra*	cultured	Tasmania, Aust.	Lleonart 2001
			South Australia	Tas. DPIWE survey
		wild	Tasmania	Tas. DPIWE survey
			NSW, Aust.	Tas. DPIWE survey
		cultured	Tasmania	Lleonart 2001, Tas. DPIWE survey
		wild	Tasmania	Tas. DPIWE survey
	H. tuberculata	wild	Channel Is. ,Britain	Clavier 1989
	H. iris	wild	New Zealand	Read 1975
P. ligni	*H. kamtschatkana*	wild	British Columbia, Canada	Horne 1996
P. monilaris[1]	*H. iris*	wild	New Zealand	Sinclair 1963
P. notialis	*H. roei*	wild	South Australia	Blake and Kudenov 1978
P. websteri	*H. diversicolor*	wild	Japan	Kojima and Imajima 1982
P. woodwicki	*H. rubra*	wild	Victoria, Aust.	Blake and Kudenov 1978
Polydora sp.	*H. midae*	wild	South Africa	Day 1967, Ruck and Cook 1999
	H. ruber, H.roei H. scalaris	wild	South Australia	Shepherd 1973
B. acus	*Haliotis sp.*	wild	New Zealand	Read 1975
B. chilensis	*H. rubra*	cultured	Tasmania, Aust.	Lleonart 2001
B. chilensis[2]	*H. iris*	wild	New Zealand	Rainer 1973, Read 1975
B. knoxi	*H. rubra*	cultured	Tasmania, Aust.	Lleonart 2001
		wild	Tasmania, Aust.	Tas. DPIWE survey
	H. laevigata	cultured	Tasmania	Lleonart 2001, Tas. DPIWE survey
		wild	Tasmania	Tas. DPIWE survey
	H. iris	wild	New Zealand	Handley 2000
	Haliotis sp	wild	New Zealand	Read 1975
B. proboscidea	*H. rubra*	cultured	Tasmania, Aust.	Lleonart 2001

[1] P. monilaris was synonymised with P. armata (Day 1954, as cited by Rainer 1973, and Read 1975)
[2] named *B. jubata* by Rainer 1973 but considered to be *B. chilensis* by Read 1975.
* Survey conducted by Tasmanian DPIWE Fish Health Laboratory 1996-1998. Identifications by M. Lleonart.

Appendix 8 A

t statistics for comparison of spionid infested &healthy abalone stocks. Foot bleed data for clin. path.

	t value	Df	p value
Cl	0.47	18	0.642
K	2.98	22	0.007
Na	-0.07	22	0.944
Na/K ratio	-5.41	22	<0.001
Ca	-0.29	16	0.772
Mg	1.37	16	0.190

Na/K ratio data arcsine transformed
Mean, SD and n values in Table 8.1

Appendix 8B

Mann-Whitney U Test statistics for comparison of mud worm infested and healthy abalone stocks. Foot bleed data for clinical pathology

	U value	p value
Cu	47.0	0.82
Glucose	6	0.13
Protein	17.0	0.66

Mean, SD and n values in Table 8.1

Appendix 8C

Long term treatment trial- 2 way ANOVA for Na/K ratio data

Source of variation	Df	Ss	ms	vr	F prob.
Time	3	97.97	32.66	3.44	0.022
Treatment	1	0.011	0.011	0.00	0.974
Time.treatment	3	30.99	10.33	1.09	0.361
Residual	60	569.66	9.49		
Total	67	698.63			

Appendix 8E

Kruskal-Wallis test and mean separation for Huon Aquaculture foot tissue protein levels

$\chi^2_{0\,05,4} = 9.488$ (Appendix Table B.1 – Zar, 1984)
20.731 > 9.488 so reject null hypothesis

Mean Separation

Comparison table by mean ranks (rank sum/n), Q = Δ mean ranks/SE

Comparison	Δ mean ranks	SE	Q	$Q_{0\,05,5}$	conclusion
Sep98 V Dec98	24.8-12.625 =12.17	6.49	1.876	2.807	Accept Ho
Sep98 V Dec99	24.8 – 9.928=14.87	5.66	2.627	2.807	Accept Ho
Sep98 V May00	24.8 – 3.75 = 21.05	6.49	3.243	2.807	Reject Ho
Sep98 V Oct00	24.8 – 23.23 = 1.57	5.09	0.308	2.807	Accept Ho
Dec98 V Dec99	12.625 – 9.93=2.69	6.06	0.445	2.807	Accept Ho
Dec98 V May00	12.625– 3.75 =8.87	6.84	1.298	2.807	Accept Ho
Dec98 VOct00	23.23-12.625=10.61	5.53	1.918	2.807	Accept Ho
Dec99 VMay00	9.928-3.75=6.18	6.06	1.020	2.807	Accept Ho
Dec99 VOct00	23.23-9.928=13.30	4.53	2.936	2.807	Reject Ho
May00VOct00	23.23-3.75=19.48	5.53	3.523	2.807	Reject Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Appendix 8F
Kruskal-Wallis test and mean separation for Aquatas foot tissue protein levels

$\chi^2_{0.05,3} = 7.815$ (Appendix Table B.1 – Zar, 1984)
13.45 > 7.815 so reject null hypothesis

Mean Separation

	Δ mean rank	SE	Q	Q 0.05,4	conclusion
Sep98 Vs feb99	18-9.2 = 8.8	3.647	2.4129	2.639	accept Ho
Sep 98Vs Nov 99	18-4.5 = 13.5	3.647	3.7017	2.639	reject Ho
Sep 98VsMay 00	18-10.3 = 7.7	3.647	2.1113	2.639	accept Ho
Feb 99VsNov 99	9.2 –4.5 = 4.7	3.647	1.2887	2.639	accept Ho
Feb 99VsMay00	10.3 – 9.2 =1.1	3.647	0.3016	2.639	accept Ho
Nov99VsMay 00	10.3 – 4.5 = 5.8	3.647	1.5903	2.639	accept Ho

$Q_{critical}$ from Table B.14 (Zar, 1984)

Appendix 8G
Seawater profile from southern study sites ($mmol.l^{-1}$)

	Ca	Mg	Na	K	Na/K ratio	Cl
	10.53	49.72	475	10.6	45	524
	10.24	53.54	451	10.4	44	534
	10.01	46.41	427	9.6	44	487
	10.2		400	8.8	45	460
	10.08	46.00	412	9.2	45	480
Mean	10.21	48.92	433.0	9.7	44.6	497.0
SD	0.20	3.50	30.2	0.8	0.5	31.0

Appendix 8H Comparative abalone oxygen consumption data

Author/date	Abalone species	Values given	Converted value	Notes
This study	H. rubra	0.7 - 1.7 μmol.g^{-1}.h^{-1}	15-35 μl.g^{-1}.h^{-1} 11-25 mg. kg^{-1}.h^{-1}	At 2 temperatures:16 & 20° C Varying degrees of mud worm infection 23-50 g abalone
Uki & Kikuchi 1975	*H. discus hannai*	2.2-3.4 ml.h^{1}.animal	39-60 μl. g^{-1}.h^{-1}	Comparison for 16.1 and 20 °C 56 g abalone
Barkai & Griffiths 1987	*H. midae*	~0.4 - 1.0 ml..h^{1}.animal	~20 μl.g^{-1}.h^{-1}	At 2 temperatures:14 & 19° C Comparison for 20-50 g animals
Nimura & Yamakawa 1989	*Sulculus Supertexta* = *H. diversicolor*	30-50 ml. kg^{-1}.h^{-1}	30-50 μl. g^{-1}.h^{-1}	Temperature unstated – but sub tropical species 40-60 g abalone
Segawa 1991	*Sulculus diversicolor* = *H. diversicolor*	600-1000 μl. h^{-1}.animal	~40 -45 μl. g^{-1}.h^{-1}	At 24 °C Comparison for largest animals 13-23 g At beginning of starvation period
Carefoot et. al 1993	*H. kamtschatkana*	0.9-1.3 ml.100g animal. h^{-1}	9-13 μl. g^{-1}.h^{-1}	At 10 °C At beginning of starvation period Data for "standard" 100 g animal
Paul & Paul 1998	*H .kamtschatkana*	40-50 μl.g^{-1}.h^{-1}	40-50 μl.g^{-1}.h^{-1}	Comparison for 15-20 ° C 45-55 g abalone
Harris 1999	*H. laevigata*	~36 mg. kg^{-1}.h^{-1}	~36 mg. kg^{-1}.h^{-1}	At 15.5 °C Total of 163 g abalone

Appendix 9A Monthly rainfall data for Dover weather station 1901-2000

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1901			127.3	185.2	80.5	77.5	64.3	49.3	95	165.9	75.7	24.4
1902	113.5	132.8	27.2	45.5	37.1	65.5	27.2	60.2	112.5	32.5	55.4	76.7
1903	42.2	94.5	74.9	70.4	65	173.5	127.8	69.6	77.2	50.3	73.2	97.8
1904	60.7	97.8	166.1	20.6	146.3	143.8	135.6	153.7	177.3			
1905	119.6	55.4	68.3	78.7	183.1	76.2	85.6	48.3	154.2	61.2	63	43.9
1906	16.5	15	75.2	94.5	58.7	117.1	94.2	56.1	22.4	188.2	69.9	19.6
1907	82	45.5	68.1	61.2	72.1	47.5	89.2	61.5	147.1	154.4	40.6	166.4
1908	31	22.1	84.6	37.3	55.6	74.4	49	50.5	61.5	126	54.4	28.2
1909	42.7	29.2	98.8	180.1	82.3	134.4	69.9	90.9	48.3	81.5	50.5	88.6
1910	87.1	23.4	48.3	88.4	72.9	127.3	70.4	74.9	128.5	75.7	49	112.8
1911	18.5	79.2	118.9	75.9	160.3	95.8	9.1	43.9	37.6	109.2	50.5	122.7
1912		2.8	48.8					84.3	133.9	97.8		64
1913	79.5	27.4	72.9	9.9	27.9	40.9	53.1	78.7	82.8	73.4	109.5	45.5
1914	7.1	17.8	38.6	115.8	10.7	42.7	60.2	63.8	52.3			
1924					46	175.1		49.8	44		84.6	
1925		37.3	87.3	126.3	83.6				48.6	86.6	22.5	
1926		46.9	28.4	95.9	66.5	83.3	110.5	114.5	72.9	133.3	79.1	
1927		76.4	37.8	16.1	87.1	141						
1945			44.2	16.9	15.6	36.3	50.3	86	115.2	49.8	29.2	27.3
1946	31.3	100.2	295.4	64.6	69.4	128.8	168.2	225.7	102.6	91.9	95.8	42
1947	31.6	41.2	104.1	26.2	98.3	173.5	138.5	98.7	73.6	250.3	34.5	96.9
1948	26.2	43.9	68.2	62.8	79.5	56.9	56.9	47.8	90.7	126	119.1	96.9
1949	104.6	104.4									118.9	
1956	69.5	53.1	64.3	66.3	147.1	180.4	70.9	104	32.4	206.7	122.3	76.6
1957	41.1	37.1	62.8	100	81	21.8	36.3	38.5	160.3	68	92.8	82.3
1958	17.1	58	90.4	47.3	243.6	88.1	84	193.9	36.9	109.4	71.7	112.8
1959	43.2	45.8	26.1	55.3	33	55	72.5	72.6	89.5	34.5	23.3	128.8
1960	32.7	28	26.4	262	133.7	34.8	66.9	55.1	72.7	74.4	69.7	8.7
1961	36.6	47.6	31.1	49.3	46.5	116.7	114.2	67.2	69.4	58.2	41.7	58.9
1962	54.5	47.7	46.5	50.7	99.2	114.7	86.1	134.2	152.8	95.3	43.8	42.1
1963	35.3	29.5	24.8	48	34.8	41.3	113.8	50.9	66.9	15.3	52.4	27.2
1964	51.7	163.4	48	34	56.9	83.8	125.2	112.9	51.6	51	89.7	134.1
1965	58.2	33.1	74.8	92.5	83.3	45.5	36.7	56.1	67.4	52.9	92	50.6
1966	20.3	24.9	58.4	101.2	60.5	18.9	115.7	54.8	90.7	97	58	27.5
1967	24.7	22	32.9	30.3	34.9	24.8	171.1	72.5	55.9	66.6	114.2	68.1
1968	16	74.9	47.5	55.5	105.7	117.3	56.2	101	103.6	89.9	157.2	43.4
1969	37.1	112.3	52.5	74.4	79.1	89.4	66.6	76.3	39.9	30.2	108.8	153.3
1970	174.4	17.4	50.9	40.8	47.9	42.3	171.6	120.2	73.4	130.9	67.8	210.3
1971	90	95.9	35.3	49.3	114.7	81.3	63.5	86	88.5	114.8	72.9	60.5
1972	34.4	52.5	29.7	73.5	29.4	60.9	152	54.5	82.6	46.6	40.1	65.8
1973	36.2	50.9	56.4	106	98.4	96.4	31.9	51.5	65	104	57.8	72.6
1974	10	45.4	55.6	73.7	77.2	94.2	187.1	68	92.4	53.2	75.6	122.4
1975	81.6	18	110.8	51.8	138.2	50.2	116.6	193.8	65.2	89.9	79	20.2
1976	117.6	11.6	55.4	41.8	60.2	58.4	57.6	142.4	84.8	68.2	118.2	133.6
1977	56.8	41.4	78.2	56	66.8	68.4	145.4	46.6	51.6	48.8	104.2	73
1978	31.2	85.6	20.4	56.4	70.2	75.6	114.4	158.4	29.8	54.6	88.4	75.4

Appendix 9A Continued....

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1979	32.2	45.8	33.2	78.4	48.4	8.2	41	91	106.6	71	40.4	78.2
1980	60	59	92.6	73.8	60.2	51.4	51	115.6	105.2	80.6	57.2	47.6
1981	25.6	13.2	108.4	113.6	43.2	121	104.6	131.8	83.8	82	34.4	70.2
1982	43.8	49	72.2	26.4	91.6	50.4	69.2	42	112.4	46.4	55	38.2
1983	48	10.4	159.6	57.6	49.6	180.2	65.6	63	167.8	58.6	143.8	22.6
1984	24.2	36.2	44.6	51.2	45.6	64.4	70.8	181.2	150.6	93.4	77.8	137
1985	84.2	57	52.4	75.8	42.8	79.6	118.8	65.4	63.6	91.8	62.2	184.6
1986	116.8	63.4	39.4	112	120.8	87.4	94.2	39.6	52.2	104.6	37.2	108.8
1987	118	44.6	77.8	25.4	70.8	44.6	57.4	54	70.2	66	67	
1988	28	10.6	17.2	21.8	75.8	88.6	99.4	75.6	82.2	182	86.4	46.4
1989	80.8	22	61	40	47.4	80.8	84.4	49.6	38	116.4	29	41.4
1990	41	61.6	39.8	26.8	72.2	48.6	204	86	31	55.6	56.4	79.8
1991	56.4	9	55.8	55.6	19.4	51.2	70.6	164	58.6	69.2	72	111.4
1992	78.6	40.8	15	48.8	55.8	41.8	125.8	83.6	87.8	49.4	90.6	53.6
1993	39	61.2	41.6	33.2	60.7	87.6	29.2	80.6	41.6	81.8	116.4	149.8
1994	57	18.4	20.2	57.2	107.4	47	75.6	118.8	111	54	112.2	10.4
1995	**72.6**	**22.8**	**75.8**	**103.4**	**36.2**	**73.2**	**139.2**	**161.8**	**69.2**	**97**	**113.4**	**96.2**
1996	100.4	152.4	98.6	179.8	11.2	55	60.4	80	104.4	79.2	101.4	49
1997	143.2	77	118.2	44.8	36	21.2	59.8	118	58.6	95.8	55.4	49.8
1998	17.2	82	21.4	51	67.6	95.8	43.8	50.2	71.8	90.4	86.8	81.8
1999	8.8	163.6	72.6	55	38.6	19.6	89.6	27.2	29.6	53.4	67.8	36.8
2000	42.8	31.4	38.4	30	84.4	49.6	108.4	82.6	86.8	114.2	28.4	100.8
2001	11.4	15.6	80.6	85.2	52.8	123						
Highest	174.4	163.6	295.4	262	243.6	180.4	204	225.7	177.3	250.3	157.2	210.3
Lowest	7.1	2.8	15	9.9	10.7	8.2	9.1	27.2	22.4	15.3	22.5	8.7
Mean	**54.7**	**52.1**	**65.7**	**68.7**	**72.8**	**79.4**	**88.6**	**87.4**	**81.6**	**88.1**	**73.6**	**76.6**
Median	42.8	45.5	55.8	56.2	66.8	75	75.6	75.6	73.5	81.5	70.8	71.4
Number	62	66	67	66	67	66	63	65	66	63	64	60